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ANALYTICAL ABSORPTION SPECTROSCOPY

Absorptimetry and Colorimetry

Edited by

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PREFACE

The present volume on absorptimetry and colorimetry has been written almost entirely from the viewpoint of what seems of most practical concern in a modern chemical testing and analytical laboratory. Thus, there are involved both the chemical problems of getting samples ready for measurement (Chap. 1) and the physical problems of testing and operating instruments in order to obtain the information desired. To the physical chemist and the physicist has been left the interpretation, or theory, of the facts upon which the analytical procedures rest.

As editor I do not presume to have resolved the long-standing confusion between chemist and physicist on the usage of terms. As an example, the term *colorimeter* is likely to mean to the physicist a tristimulus kind of matching instrument (Chap. 9), whereas to most chemists it means a device for comparing the lightness (intensity) of one color with another. If one considers comparimetry (Chap. 3) as a kind of relative absorptimetry, perhaps it is feasible to restrict colorimetry to the meaning implied by the physicist.

Intentionally the material included has been confined to the spectral regions usually implied by the terms *ultraviolet*, *visible*, and *infrared*. For the kinds of instruments described, this limits the wavelength range to about 0.2 to 25 microns.

I have assumed responsibility for the general outline of the book. With this over-all objective in mind, each contributor was requested to present, in the space available, a balanced, contemporary statement of topics he considered relevant to his respective assignment. In the subsequent editorial coordination of this material, the chief aim has been to keep at a minimum inconsistency in terms and symbols, and to avoid unnecessary overlapping in the various chapters.

The artificial and overemphasized distinction between organic and inorganic chemistry has been almost entirely disregarded. To the analyst, carbon (element No. 6) is just one of the now known ninety-seven elements with which he has to deal, either as such or in the form of radicals or compounds. Each element has its characteristic chemistry, in terms of which analytical methods must be devised and operated. If one accepts this viewpoint, there is no such thing as organic or inorganic spectrometry.

On behalf of the several contributors I hereby express their thanks, together with my own, to all the different organizations which so willingly and generously provided illustrative material.

The individual contributors and I will appreciate reports of any errors noted or suggestions for improvement of the book.

M. G. MELLON.

Lafayette, Indiana,
January, 1950.

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1· CHEMISTRY

Preparation of Systems for Absorptimetric Measurement

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A·INTRODUCTION

The chemistry of an analytical method is found almost entirely in the succession of operations required to prepare samples of many different kinds of materials for measurement. To the extent that chemistry is involved, it consists in general of certain preliminary transformations and separations, often combined in absorptimetry with the development of a suitably colored system. Thus, a sample may require one or more of at least the following operations: fusion, dissolution, reduction, oxidation, change in *p*H, and complexation. Even more involved may be the separation either of the desired constituent or of interfering constituents by methods such as volatilization, precipitation, electrodeposition, and extraction.

Explanation is given later for the definitions and limitations used for the terms *absorptimetry* and *colorimetry*. It will suffice at this point to note that our interest is the analytical application of the absorption of radiant energy in the spectral region of 0.2 to 25 microns, the range commonly designated as the ultraviolet, visible, and infrared.

1·1 The problem. From the standpoint of the analyst, colorimetry includes those procedures dealing with the determination of a desired constituent by measurement of some property related to color. Recent progress in the development of colorimetric methods has resulted largely from application of the photoelectric cell to the problem of color measurement. This improvement eliminates entirely the subjective difficulties which invariably complicate visual color comparison. As a result of such advances in instrumentation, the sensitivity and accuracy obtainable with many colorimetric methods have been greatly increased. It is noteworthy, however, that the development of improved methods, based on new chemical reactions or a better understanding of old ones, is a somewhat neglected field. The chief limitations of most procedures exist in the chemical reactions on which they are based rather than in the instruments available to the analyst, as the best modern color comparators, filter photometers, and spectrophotometers more than fulfill most of his present needs.

To the physicist, colorimetry is measurement of color as such (see Chapter 9), and therefore involves no chemistry. In chemical analysis, on the other hand, one seldom finds the system originally in a condition suitable for colorimetric measurement. To determine manganese in steel, for example, the sample must first be dissolved. The manganese is then converted to a form having suitable colorimetric properties. If other constituents interfere with the color reaction or measurement, they must be removed, compensated, or prevented from functioning through appropriate chemical treatment.* These transformations constitute the chemical portion of the analytical procedure.

The scope of colorimetry is growing at an accelerated pace and is no longer confined to isolated determinations of trace constituents. Hedin has recently published methods for analysis of silicate rocks requiring only eight hours for completion (*215*). All constituents except calcium are determined colorimetrically, although calcium could have been included with the rest. Systematic schemes have been devised for colorimetric analysis of soil and plant materials by various workers (*369*, *383*, *395*) and for several metallurgical materials by Haywood and Wood (*214*).

Most colorimetric determinations are carried out on liquid solutions, although colored gases such as nitrogen dioxide may be measured as such, and a method involving measurement of color produced by carbon monoxide on a solid absorbent is now in use.

Given a suitable reaction, a colorimetric method may be devised, provided the intensity of the coloration produced is essentially dependent upon the amount of desired constituent present and is stable long enough to permit its measurement. Colorimetric methods, not being limited to elements, are frequently applied to the determination of compounds or radicals as well. Consequently, the different possibilities far exceed those for emission spectrometry. The desired constituent may be colored or capable of forming a color with certain reagents, or it may affect the extent to which some other color reaction proceeds. An example of the second type, of which there are relatively few, is the determination of fluoride based on its interference with the titanium-peroxide or iron-thiocyanate reactions. A few constituents which do not undergo color reactions themselves are determined indirectly through precipitation as insoluble salts with other chemically equivalent ions for which methods are available. Alkali and alkaline earth metals are commonly determined by such indirect methods. These metals, which do not exhibit pronounced coordinating tendencies, cannot be determined by the usual organic reagents which have been so successfully applied to the transition elements and heavy metals. Recent work on qualitative reactions of lithium, calcium, and magnesium with a number of organic compounds, however, indicates the possibility of eventually developing suitable reagents for the alkalies (*275*).

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* In accordance with the definitions of terms given in Chapters 5 and 9, various expressions in this discussion, such as *color*, *colorimetry*, and *color-forming*, all refer to systems absorbing radiant energy selectively in the visible region of the spectrum. The absorption of radiant energy by many substances, however, is not limited to this region, and subsequent chapters deal with measurements covering the spectral range 0.2 to 25 microns. Thus far the use of reagents to develop systems absorbing in the ultraviolet or infrared regions is practically unknown. To the extent that such reactions should prove possible, the discussion of this chapter may be extended. Systems possessing color represent merely a part of the larger field.

Colorimetric determination of alkali metals (or alkaline earths) in mixtures with each other usually depends upon separations based on differences in solubility among the various metals in the group. Such methods are not generally desirable, as they lack both selectivity and sensitivity. Quite frequently the concentration to be determined is close to the solubility limit of the compound to be precipitated.

In the organic field one encounters a problem similar to the analysis of mixtures of elements in the same periodic group. It is often desired to determine low concentrations of one or more compounds in a homologous series in the presence of one another. Separations by the conventional methods are usually out of the question, as the amounts present are too low to permit ordinary losses. Occasionally, there is sufficient difference among the reactivities of the lower homologs to distinguish them in mixtures, as in the determination of formaldehyde in the presence of acetaldehyde with Schiff's reagent. The few reactions peculiar to the first member of a series may be exploited, if the occasion permits; but there are too few opportunities of this kind in everyday work.

The biochemist is aware of this problem in his dealing with various natural products. They may be closely related chemically and measurable by the same analytical procedure although quite dissimilar in their biological activity (*514*). In the irradiation of certain oils to increase vitamin D content, for example, various products having no vitamin activity may be formed. Since these products react with the antimony chloride used for determining vitamin D and are reported as such, the limitation of the method imposed by its lack of specificity is readily apparent.

Many industrial processes yield small amounts of derivatives of the main product or closely related products for which analytical methods are needed. Colorimetric methods are ideal from the standpoint of simplicity and speed and are usually capable of sufficient accuracy for the purpose. It is not always that a satisfactory reaction is available for the job, however, and, as in the analysis of systems containing members of the same periodic group, development of a method must await discovery of new and more selective reactions.

1·2 Chemistry and Beer's law. The laws of absorption of radiant energy, as they are concerned in the measurements of analytical chemistry, are discussed in Chapter 2. It is noted there that factors responsible for deviations from Beer's law may be either chemical or physical. Although the relationship of chemical composition to the validity of this law might consistently be included here, this discussion has been put under the general presentation of the laws of absorption.

B·PREPARATION OF THE SAMPLE

Every analyst knows that all too frequently the most difficult and exacting phase of his work is not the final measurement but the preparative treatment to which the sample must be subjected. By preparative treatment is meant those operations by which the desired constituent is obtained in a form suitable for measurement. Included are any such steps as ashing, dissolution of the sample, concentration, separations, and elimination of interference by various chemical means.

1·3 Preliminary treatment. The appropriate preliminary treatment for obtaining a solution of the desired constituent is determined by the nature of the sample, the constituent to be determined, the method of measurement, the incidental constituents, the desired accuracy, and the time available. The nature of the sample is probably the major consideration. Frequently, the easiest and simplest method of attack is the best one. High-temperature ignition, for example, may entail loss or render the ash difficult to dissolve. A simple extraction, on the other hand, may give excellent results. Iron and copper in pentaerythritol may be determined by merely dissolving the sample, acidifying, adding the necessary reagents, and measuring the color. This procedure saves considerable time over the long ashing otherwise required (*350*).

Dry ashing of samples should always be carried out at a temperature as low as possible, and the operation is necessarily a slow one. Considerable patience and experience may be required to achieve a good ashing job. A few typical examples will illustrate the methods available for ashing or otherwise getting the sample into suitable form.

The determination of lead in spray residues left on the surface of apples may be carried out after extraction with dithizone in chloroform (*531*). No acid digestion or ashing treatment is required.

In the determination of inorganic salts in crude oils, extraction by refluxing the sample with dilute hydrochloric acid gives excellent results (*399, 400*). The same treatment may also be used on certain liquid terpene products, which are troublesome to ash directly (*350*), and probably on a wide range of other liquids insoluble in dilute acids.

An automatic device for ashing organic materials such as lard, which is very difficult to burn without losses, has been developed recently (*293*). The liquefied sample is allowed to flow dropwise into a heated crucible at a controlled rate such that no flashing occurs although the sample is completely decomposed, leaving only the ash in the crucible. Both the sample reservoir and crucible are electrically heated. A 200-gram sample may be ashed in 8 hours with a minimum of attention. Metals in the ash are then determined after fusion with potassium pyrosulfate.

Ores and metals may be dissolved by conventional means, usually acid treatment (*308, 314, 315*). Silicate rocks are generally decomposed by the classical fusion methods (*215, 519*), although Sandell determines tungsten after treating the sample with hydrofluoric, sulfuric, and nitric acids (*423*). The R_2O_3 group is precipitated with sodium hydroxide, and molybdenum is then precipitated in the filtrate as the sulfide and collected with antimony sulfide. Tungstate in the filtrate from that separation is determined as the thiocyanate complex, using chlorostannous acid as reducing agent, and separated ready for measurement by one extraction with diethyl ether.

Biological materials are best decomposed by wet digestion. Nitric, sulfuric, and perchloric acids used in order offer a superior method of decomposing a variety of samples. Only a small amount of perchloric acid is usually required, since its action seems to decompose the organic materials into simpler compounds oxidizable with nitric acid. Since nitric acid is the chief oxidizing agent, the temperature should be kept low enough to maintain a high concentration. Considerable information on the subject of decomposing organic and biological samples in this way has been published (*80, 240, 369, 383*).

Jackson, in determining iron with 2,2′-bipyridine, studied various methods for ashing biological samples and also concluded that the only acceptable method was to use nitric, sulfuric, and perchloric acids (*240*). Dry ashing with a small amount of sulfuric acid, with sodium carbonate, or with calcium carbonate gave poor results even on synthetic mixtures of iron with glucose, urea, calcium phosphate, and sodium chloride.

In the determination of nitrogen the Kjeldahl method of digestion has been used, without distillation, for decomposing biological materials. Nitrogen is determined directly in the sulfuric acid digest by the Nessler method without interference by selenium, although sulfuric acid must be added to the standards (*340*).

For the determination of metals in cellulose and cellulose derivatives, good results are obtained by decomposing the sample with nitric acid (*436*). Large Vycor beakers are inexpensive, durable, and convenient for this purpose, since large samples can be treated in one portion. With the possible exception of silica, contamination is no more a problem than if platinum is used. Sulfuric acid requires somewhat less time than nitric acid in decomposing linters but is ineffective with cellulose nitrate. After digesting the sample with nitric acid, the excess acid is driven off by heating over a flame, and the carbon burned completely in a furnace at 550°C.

Detailed information on how best to effect the preliminary treatment of even a representative selection of sample types is beyond the scope of this book. Those examples cited will serve to bring out some of the more important principles. Detailed procedures for many materials are available in standard sources and are helpful as a general guide in developing new or improved methods.

1·4 Separation and concentration of trace constituents. When the desired constituent is present in the sample at a concentration too low for direct measurement, one must resort to appropriate means of bringing the concentration up to the sensitivity limits of the method. If this increases the concentration of other constituents sufficiently to cause interference, a separation may then be necessary. Various separations used only as means of eliminating interference are discussed subsequently under that heading. For the present we shall be concerned primarily with separations which are incidental to the processes used in concentrating the desired constituent.

Generally speaking, an analytical separation is an operation designed to take advantage of a phase change produced by chemical treatment of a homogeneous system. Separations may involve precipitation, extraction, or volatilization (*317*). Electrodeposition, chromatography, and ion-exchange separations are regarded by the author as different forms of precipitation, although the methods and apparatus may deviate considerably from those used in the more familiar precipitation procedures.

1·5 Precipitation. If the desired constituent is present in a very low concentration (less than 0.01 per cent), precipitation and handling of such a small amount are usually avoided if possible. Losses due to incomplete precipitation and difficulties in the manipulations required can be overcome, however, if a suitable collecting or gathering agent is available. The collecting agent should be sufficiently insoluble that only a small amount is required and in general should be flocculent rather than granular or crystalline. The action of such materials may result from mechanical inclusion or entrainment, coprecipitation, mixed crystal formation, general (as opposed to columnar) adsorption, or shortening of the time required for attainment of equilibrium in the precipitation (*414*, *537*).

Usually the collecting agent is precipitated in the presence of the desired constituent, although the separation is sometimes effected by passing a solution of the sample through a filter of paper or asbestos impregnated with the collecting agent. A few typical examples of collecting agents for metals are listed in Table 1·1.

TABLE 1·1 COLLECTING AGENTS FOR METALS

Metal	*Collecting Agent*	*References*
Bismuth, indium, iron	Hydrous manganese dioxide	*537*, *557*
Mercury, iron	Cadmium sulfide	*20*
Molybdenum	Antimony sulfide	*20*
Copper	Lead sulfide	*20*
Lead	Calcium phosphate	*20*
Lead, arsenic	Hydrous ferric oxide	*20*
Gold, silver	Mercury	*414*
Titanium	Hydrous aluminum oxide	*414*
Molybdenum, zinc, lead, mercury	Copper sulfide	*414*
Zirconium, vanadium, titanium	Iron cupferrate	*414*
Gold, platinum, palladium	Tellurium	*414*
Aluminum	Ferric phosphate	*86*

Electrodeposition and adsorption methods are valuable in handling a large number of samples of similar nature where conditions can be standardized. For occasional determinations on research samples varying in type and in minor constituents, such as alloys, resins, plastics, and organic compounds, the other, more versatile means of separation are more convenient. Electrodeposition using the mercury cathode has received emphasis recently and is capable of making clean separations without contaminating the sample (*246*). Ordinarily, it is preferable to deposit the undesired constituents in the mercury. Special apparatus for mercury-cathode separations is now available commercially.

Among the most interesting methods of performing analytical separations are the differential or columnar (chromatographic) adsorption and ion-exchange processes. Although these are similar with respect to apparatus and basic principles, they differ somewhat in the types of materials which can be retained on the column. Essentially, ion exchange and chromatography are types of precipitation employing an insoluble, immobile precipitant. Ion exchange differs from chromatography in that it imparts to the solution certain ions in exchange for those which it retains, whereas the chromatographic type of adsorption is not limited to reaction with ionized compounds.

The columnar adsorption method is used ordinarily on organic compounds which are relatively nonpolar, although certain carboxylic acids of low molecular weight can be separated by partition chromatography using alumina to support the immobile, aqueous phase. Some of the higher fatty acids are also separable by straight selective adsorption from ether solution.

An interesting development in the available methods of separating metals is the application of chromatography to the dithizone complexes (*142*). With carbon tetrachloride as solvent, antimony (III), tin (II), nickel (II), and manganese (II) are completely adsorbed on alumina. Other bands occur in the following order: copper, iron, excess dithizone, cadmium, cobalt, zinc, and mercury.

Ion exchange, as the name implies, is a metathetical reaction between ions in solution and the exchange medium, usually a granulated resin containing basic or acidic groups which are capable of retaining the dissolved ions on its surface and liberating them later after the desired separation has taken place. Under equilibrium conditions, the exchange reactions are governed by the law of mass action. Of greatest interest to the analyst are the cation-exchange resins which are used for separating the metallic ions.

The extent of retention of a particular cation is determined largely by its valence and its radius when hydrated. The cation-resin bond strength increases with charge on the cation and decreases with ionic radius. A typical series, in order of decreasing bond strength, is as follows: thorium, lanthanum, cerium, rare earths, yttrium, barium, cesium, strontium, potassium, ammonium ion, sodium, hydrogen (*46*).

Separation of cations involves first adsorbing the mixed cations on the resin column and then selectively eluting the desired cation from the resin by washing with suitable reagents, preferably an elutriant which will form a soluble and relatively stable complex with the desired constituent and leave the undesired cations on the column. By varying such conditions as *p*H, stability of the complexes can be altered so as to effect desired separations. For example, zirconium and columbium are removed from Amberlite resin with oxalic acid, leaving all bivalent and tervalent metals. Citric acid may then be used at *p*H 3 to elute the tervalent metals and at *p*H 5 to elute the uni- and bivalent metals.

In the separation of cadmium from zinc, manganese, nickel, and copper with Amberlite resin, 0.1 *N* hydrochloric acid may be used to elute the cadmium and leave the other metals, whereas all are removed with 1 *N* acid (*397*). The same resin has also been used for removing calcium from asparagus juice in the preparation of standards for spectrographic analysis (*131*) and for separating copper from milk before its polarographic determination (*107*). Ion-exchange resins have also been used in the separation of amino acids on a semiquantitative basis (*92*, *482*).

8-Hydroxyquinoline has been used as the exchange medium in ion-exchange separation of the metals (*143*). In addition to having an exchangeable hydrogen, this reagent possesses complexing properties which should make it a highly selective filling for the exchange column.

Considerable work on the theory of ion exchange has been reported (*14*, *28*, *45*, *255*, *273*), and an increasing variety of apparatus and exchange media is commercially available (*325*). This technique appears to be rapidly assuming an important position in general analytical practice. Noteworthy application of this principle is found in the separation of the rare earth metals and of atomic fission products (*212*, *302*, *479*, *480*, *481*, *506*).

1·6 Extraction. Extraction by a partition or liquid-liquid process is frequently the most convenient and effective method of concentration and is capable of giving good results with a minimum of manipulations if the partition relationships are favorable. The volumes of solutions are usually large enough that significant losses may be easily avoided, and, if necessary, the extracts can always be further concentrated by evaporation.

In the determination of aluminum in biological material, Cholak, Hubbard, and Story add iron to entrain aluminum as the phosphate and then remove iron by extraction as the cupferrate in a benzene-ether mixture (*86*).

A few inorganic compounds are extractable from aqueous solution by organic solvents, for example, chlorides of iron and gold by diethyl ether. Metals are usually converted to water-insoluble complexes with organic reagents in order to make the extractions. Many of the metal complexes are nonelectrolytes insoluble in water but readily soluble in organic media. Nickel, for example, may be extracted from aqueous solution with dimethylglyoxime in chloroform. Extractions involving dithizone are in wide use and hardly need discussion at this point. Detailed information concerning the theory and procedures for dithizone may be found in other sources (*414*).

Extraction of certain metals as their complexes with 8-hydroxyquinoline (oxinates) has received attention recently. By controlling *p*H it is possible to separate metals in much the same way as is done with dithizone. Table 1·2 lists some of the metals which can be extracted by this method, using chloroform as solvent, and the wavelengths recommended for colorimetric measurement.

TABLE 1·2 EXTRACTION OF METALS AS OXINATES

Metal	*p*H	*Wavelength, mμ*	*References*
Aluminum	4.5, 8.5–11	395	*174, 332*
Indium	1.9–4.5	400	*331*
Iron	1.9–3.0	470, 570	*332*
Bismuth	4.0–5.2	395	*332*
Cobalt	6.8	420	*332*
Nickel	6.7	395	*332*
Copper	2.7–7.0	410	*332*
Vanadium	4.0	*	*415*

* Tungstiphosphoric acid reagent is used for the color measurement in the case of vanadium because of iron interference if the brown oxinate complex is measured. Iron also interferes in the determination of aluminum but can be extracted at *p*H 2, leaving aluminum in the water layer.

The long-known process of dissolution may be considered a liquid-solid extraction. Separation of sucrose from sand in this way is a simple example. Many others are common in the agricultural, food, and pharmaceutical industries. When one contemplates the action of even the common solvents on all the solid materials daily subjected to analysis, the separative possibilities are vast in number.

Absorption, as a separative operation, is usually a gas-liquid or a gas-solid process. It is most familiar in the methods of gas analysis. With a solid such as steel, the carbon dioxide to be absorbed is first formed by volatilizing the carbon in a stream of oxygen. In this case the over-all method involves both volatilization and absorption processes.

1·7 Volatilization. Distillation methods of separation and concentration are often used in the determination of organic materials not possessing characteristic reactions that can be utilized for separations.

With inorganic compounds, distillation is less useful, although certain nonmetals and metalloids can be distilled readily in the form of their halides. The Kjeldahl method, of course, is famous as a means of separating nitrogen as the hydride.

Arsenic can be separated from phosphate by distillation as the trichloride (*80*) or as the pentabromide (*297*). Arsenic, antimony, and tin may be separated from each other by distillation of the chlorides, arsenic coming over at 110°C., antimony at 155–165°C., and tin at 140–150°C. after adding hydrobromic and hydrochloric acids (*537*). Phosphoric acid is added to hold back the tin during distillation of the antimony.

Germanium is distilled as the tetrachloride from hydrochloric acid solution (*257*). Boron may be isolated for measurement or eliminated as an interferent by distillation as the methyl ester of boric acid. Osmium is distillable as the tetroxide from nitric acid (*422*).

Thus far consideration has been given only to systems in which the desired constituent is condensed and recovered. The process is generally known as distillation.

Volatilization is not limited to such systems. In fact, they may be a minority of the total possibilities. The separation of carbon from steel as the dioxide has been mentioned. The determination of moisture by drying and the "loss on ignition" of limestone are examples of a very general type of application. The general subject of analytical separations by volatilization seems to be especially in need of critical review and further study.

1·8 Elimination of interference. Few reagents used in colorimetry are specific for any one constituent, although this situation—a specific reagent for any desired constituent—would be highly desirable. Because of the subtle nature of many chemical reactions on which colorimetric methods are based, the analyst must anticipate those factors likely to lead to interference and know how to deal with them if their effects are serious. By interference, as distinguished from other types of errors, one usually means the action of some undesired constituent to cause erroneous results. Interference may result from (1) conversion of the desired constituent or the color-forming reagent to an unreactive product by such reactions as oxidation-reduction, precipitation, or complexation; (2) reaction of the colored product with a constituent present, resulting in fading; or (3) development of an extraneous color or turbidity by the reagent, desired constituent, or other materials present.

The general applicability of a color reaction for analytical purposes depends largely on the availability of methods for circumventing the effects of interfering constituents occurring in the sample. These methods of dealing with interference usually involve one or more of the following principles:

A. Separation.
B. Chemical transformation to noninterfering products.
C. Compensation.
D. Use of monochromatic or filtered light (for interference due to color).

1·9 Separation. Although separation of the desired constituent from interfering substances is usually one of the first expedients considered, it is generally an undesirable complication and should be employed only as a last resort. In addition to being time-consuming, it increases the possibility of contamination and loss. The general methods of effecting separations are discussed in sections 1·5, 1·6, and 1·7.

1·10 Chemical transformation to non-interfering products. The most desirable method of eliminating interference is chemical in nature and involves processes for converting the interfering constituents into forms which do not influence development of the color. Success in developing useful methods for accomplishing this end requires a thorough knowledge of chemical reactions, and it is not surprising that this field has been in considerable neglect. Even though chemical analysis in general stands to profit from this type of work, most investigators seem to concentrate on the more immediate and specific type of problems.

Reactions which have been utilized for eliminating or reducing interference include the following types: (1) oxidation-reduction; (2) complexation and decomplexation; and (3) miscellaneous reactions, such as hydrolysis, saponification, and condensation. The following discussion is devoted to typical means of eliminating interference by these *in situ* chemical methods. Although there are deviations from the above classification, it does summarize a few of the more interesting and ingenious examples of what has been done in this direction. Other applications of these principles will probably occur to the reader.

1·11 Oxidation-reduction. In the determination of riboflavin in dried milk products, instable colored impurities are removed by controlled oxidation in the procedure of Sullivan and Norris (*493*). Riboflavin is then measured at 445 mμ and reduced to the leuco form with hyposulfite (dithionite). The residual color is then measured and subtracted from the initial value.

Carruthers, in the determination of ascorbic acid with 2,6-dichlorophenolindophenol, used mercuric chloride to prevent reduction by other substances such as glutathione (*72*).

In the determination of phosphate in boiler water, tannin interferes because of its color and by preventing coagulation of suspended matter. Goldman and Love studied methods of dealing with this interference and found bleaching by oxidation with potassium peroxydisulfate to give excellent results and a colorless blank even with large amounts of tannin (*182*). Removal of tannin on decolorizing carbon was also recommended.

Hill (*219*) recommends potassium nitrite in preference to hydrogen peroxide for reduction of manganese dioxide in the determination of phosphorus in steels as the molybdivanadiphosphate. Sodium molybdate and metavanadate, incidentally, are used to avoid formation of the "yellow precipitate" which results if ammonium salts are used.

Tiron, an interesting reagent reported by Yoe and coworkers, may be used for the simultaneous determination of titanium and iron (*560*, *565*). It is the disodium salt of 1,2-dihydroxybenzene-3,5-disulfonate. Iron is measured as the ferric complex at 560 mμ and reduced with sodium dithionite before measurement of titanium at 410 mμ.

1·12 Complexation and decomplexation. Silicon in copper-base alloys may be determined as the molybdisilicate complex by using hydrofluoric acid to dissolve the sample. Both silicic and fluosilicic acids react to give the complex. Boric acid is recommended by Case to destroy excess hydrofluoric acid by converting it to the fluoborate (*74*). Citric acid is added to destroy the colored molybdate complexes of phosphorus, germanium, and arsenic. The molybdisilicate complex, being more stable, is destroyed more slowly and can be measured separately.

Parri, in the determination of phosphorus by the molybdate method, also eliminates fluoride interference by boiling with boric acid before developing the color (*370*).

In the determination of silicon in magnesium and light alloys, Boyle and Hughey use ammonium tartrate to tie up aluminum, iron, and zinc in stable complexes (*48*). Rozental and Campbell use sodium fluoride to decolorize iron and phosphorus compounds in determining silicon in steels (*409*). Correction for chromium is made in the calibration. Straub and Grabowski recommend oxalic acid to remove phosphate interference in the determination of silica in water (*492*).

Fluoride interference may be avoided in the determination of phosphate, as well as in the determination of silicate, by treatment with boric acid (*258*, *274*). Wirth, in the determination of meta- and pyrophosphates, uses aluminum to displace iron from phosphate complexes before measuring iron thiocyanate colorimetrically (*546*). The same treatment is employed in the A.S.T.M. method for iron in water, using 1,10-phenanthroline (*7*).

Schulek and Floderer studied the reaction between ferrous iron and bipyridine in considerable detail, and several interesting features are incorporated into their procedure for iron in pharmaceutical materials (*437*). Development of the color is carried out in the dark, although the reduction step, required for total iron, is performed in the presence of light. Phosphoric acid is added, when ferrous iron is determined directly, to reduce interference which, according to the authors, results from presence of ferric iron. Potassium fluoride is also used to tie up ferric iron, and potassium cyanide is added to eliminate copper interference. Anions such as cyanide and phosphate, which form stable iron complexes, retard development of the color. It is necessary, therefore, to allow 24 to 48 hours before making the color measurement.

Where it is desired to eliminate interference of the tervalent metals, such as iron, aluminum, and chromium, it is well to consider converting them to such complexes as the phosphate, fluoride, oxalate, citrate (*373*), and tartrate (*298*). These are relatively stable and allow only a very low concentration of the metal ion at equilibrium. Brabson, Schaeffer, Truchan, and Deal use ammonium citrate to prevent precipitation of aluminum phosphate in the determination of copper and iron in red phosphorus (*51*). Citrate ion interferes appreciably in the iron determination, but this can be avoided by adding an excess of reducing agent and phenanthroline, the color reagent.

In the determination of copper in yeast and beer, Stone makes use of several interesting principles (*489*). The color is developed with sodium diethyldithiocarbamate and extracted with amyl acetate. Bipyridine is added to retain the iron, which is in the ferrous state, in the aqueous phase. The method gives excellent results.

Sodium diethyldithiocarbamate is useful not only as a color reagent for copper but also as a masking (complexing) reagent to tie up other metals. Cowling and Miller, in the determination of zinc in plant materials with dithizone, eliminate interference of practically all other metals which form dithizone complexes by adding dithiocarbamate (*105*). The color is extracted ready for measurement in carbon tetrachloride. Because of incomplete extraction, there is some loss in sensitivity for zinc and a deviation from Beer's law. The mixed-color method of measurement is recommended, since lead is present in the carbon tetrachloride extract as the dithiocarbamate and is converted to the dithizonate on shaking with dilute ammonia, as required in the final step of the one-color method.

Serfass and Levine circumvented this latter difficulty by using sodium bis(2-hydroxyethyl)dithiocarbamate to hold back copper, cadmium, nickel, and lead, thus making theirs a one-color procedure (*449*).

According to Cholak, Hubbard, and Burkey (*84*), di-β-naphthylthiocarbazone offers a distinct advantage over dithizone, to which it is analogous, in the zinc determination. It is particularly recommended when dithiocarbamate is used as a complexing reagent to hold back other metals. According to the authors, substitution of this reagent for dithizone (1) eliminates certain partition losses which occur with dithizone in the *p*H range 8.3 to 10.5, (2) eliminates other losses resulting from the effect of dithiocarbamate, (3) effects conformity of the colored system to Beer's law, and (4) permits use of dithiocarbamate in the initial extraction step so that zinc can be separated immediately in sufficient purity for either colorimetric or polarographic determination.

For the determination of antimony in biological materials, Rhodamine B is used as the color-forming reagent in Maren's procedure (*301*). If nitric acid is used for digestion of the sample, antimony tetroxide is formed and must be reduced with sulfur dioxide and then oxidized with ceric sulfate. With perchloric acid, however, both these steps are eliminated. Chloride ion is essential to development of the color and is added as hydrochloric acid. Iron interference may be reduced by adding phosphoric acid, but, since this inhibits the color reaction on prolonged standing, the determination should be concluded as rapidly as possible.

A separation of antimony (V) from iron (III) may be effected by extraction with isopropyl ether from a solution 1.5 *N* in hydrochloric acid. This leaves 90 per cent of the iron in the aqueous phase. The color can then be formed by shaking the extract with an aqueous solution of the reagent, the complex remaining in the ether layer. Strangely, the complex does not go into the ether if formed in aqueous medium, although it can be extracted with benzene and other solvents.

A striking example of how to deal chemically with a difficult problem in interference is shown by the work of Putsché and Malooly on the determination of cobalt in stainless steel by the thiocyanate method (*389*). Excellent results over the wide range of 0.2 to 44 per cent are reported by their procedure. The following constituents may be present in the sample: iron, chromium, nickel, manganese, copper, titanium, aluminum, molybdenum, vanadium, tungsten, columbium, and several other metals and nonmetals.

The sample is dissolved by appropriate treatment with hydrochloric, nitric, hydrofluoric, and perchloric acids. Iron is separated with zinc oxide or by ether extraction in the form of its cupferron complex. Reduction with chlorostannous acid is also effective in reducing iron interference. Sulfurous acid is added to reduce any iron and chromium left, and a red filter combination eliminates interference by any residual ferric iron surviving the chemical treatment. Two color measurements are made in each determination, one in water and one in acetone medium. The difference between these two measurements, corrected for their respective blanks, is a measure of the cobalt, its thiocyanate being blue in water (pink on dilution) and blue in acetone.

1·13 Miscellaneous reactions. Hydrolysis of an interfering constituent to a noninterfering product is utilized in Jackson's bipyridine method for iron in biological material (*240*). The sample is decomposed with sulfuric acid and finally ashed at 550°C. This treatment favors formation of pyrophosphate, which is a source of interference in the bipyridine method. The recommended procedure is to hydrolyze the pyrophosphate to orthophosphate by heating the ash with hydrochloric acid, after which the color can be developed fully in a few minutes without interference.

Hydrolysis is also used in a different way in eliminating interference in Ford's procedure for penicillin (*161*). This method involves colorimetric measurement of the ferric complex of the hydroxamic acid formed by reaction of the desired penicillin with hydroxylamine. This reaction is not specific, however, since it takes place with many esters, anhydrides, and amides. In order to correct for interference from these sources, a separate determination is made after hydrolyzing the penicillin to penicilloic acid by action of the enzyme penicillinase. Being specific for penicillin, the enzyme does not attack the various possible interfering constituents, and they can be measured to establish the appropriate correction after destroying the penicillin.

With colored systems which are relatively unaffected by variations in *p*H it is possible to reduce interference from certain sources by proper control of this variable. In the determination of iron with 1,10-phenanthroline, for example, interference by copper is minimized at *p*H 4. Beryllium complexes with the reagent below *p*H 3 and precipitates above *p*H 5.5. Between these limits, the effect of beryllium is negligible (*163*).

In the antimony trichloride method for vitamin A in fish-liver oils, Oser, Melnick, and Pader used a saponification treatment to destroy certain inhibitors (*363*, *364*). An "increment" procedure was also recommended and will be taken up in the next section under compensation methods. Water interference in the determination of vitamin A in milk is eliminated by adding a drop of acetic anhydride before the antimony chloride (*47*).

Wolf uses formaldehyde to tie up ammonia in the determination of potassium in soil and plant extracts with sodium cobaltinitrite reagent (*547*). A photometric measurement with a 650-mμ filter is then made.

Another example in which an organic synthesis is employed in eliminating interference is the determination of vitamin D by the method of Milas, Heggie, and Raynolds (*326*). Treatment with maleic anhydride destroys vitamin A, carotenoids, and other materials, except vitamin D, which react with antimony trichloride, the color-forming reagent.

In the Schechter-Haller procedure for determining DDT (*432*), significant losses occur if a small amount (microgram quantities) is nitrated in the presence of large amounts (200 mg.) of fruit or vegetable waxes in developing the color. If oleic acid (10 mg.) is added, results are close to theoretical, according to Clifford (*93*). In the concentration range corresponding to spray residues, this difficulty is not encountered. The nature of this "protective" action provided by oleic acid in reducing the decomposition of small amounts of DDT is unexplained.

The foregoing examples are typical of what has been done in the improvement of certain colorimetric procedures. They emphasize the need of a broad chemical background on the part of the analyst and a familiarity with many types of reactions if he is to be successful in transferring his methods from standard solutions to the more complex samples of everyday life.

1·14 Compensation. Interfering constituents may be compensated either internally or externally. By internal compensation is meant addition of measured increments of the desired constituent to the sample so that the calibration, in effect, is based on standards containing the sample. Uncertainties arising from the presence of other constituents, turbidity, extraneous color, and other sources, except contamination involving the desired constituent in the reagents, are compensated. External compensation refers to addition of the interfering constituent to the standards and blanks so as to simulate the composition of the sample. Obviously, one must know the approximate amount present in the sample in order to apply this latter method. If the interference is due to a colored constituent whose color is merely superimposed on the final color developed, it would suffice to add the same amount to the blank solution used to adjust the instrument to zero. An alternative method would be to subtract a concentration equivalent to the color contributed by the interference. If there is a chemical interaction, however, the interfering substance should be present also in the standards.

1·15 Internal means. The internal method of compensation, or "increment procedure," has been used to good advantage in vitamin assay work, where interfering constituents present a difficult problem and separations are frequently out of the question. The principle involves calculation of the result from addition to the sample solution of known increments of the compound being assayed, instead of referring to a calibration curve established with pure standard solutions. Oser, Melnick, and Pader discuss the method with respect to vitamin A work and give references pertaining to prior use of the principle (*364*). It is assumed that the vitamin added to the sample solution is subjected to the same inhibitory (or accelerating) conditions during the color development as the vitamin originally present. A linear relationship exists between the amount added and the color developed, although the color may deviate considerably from that obtained with standard solutions. According to Oser and coworkers, the increment method is the best way to compensate for such variables as concentration, presence of water and alcohol, reagent instability, and effect of temperature.

These authors corrected for turbidity by making a transmittancy measurement at 720 mμ where the colored product does not absorb. The effect of carotene, which also gives a blue color with the antimony trichloride reagent, was taken into account by a second color reading after 2 hours. The initial vitamin A color, which is read at 4 seconds, has faded completely at the end of 2 hours, leaving only the color due to the carotene.

Another application of the increment method was made by Lamb (*280*) and by Melnick *et al.* (*322*), in the determination of nicotinic acid and nicotinamide. Here, again, the calculation involves the increment in absorbancy obtained with a known addition of the desired constituent to an aliquot of the sample.

A simple calculation involving internal compensation has been used in the determination of copper with 1,10-phenanthroline (*348*). Instead of reading the unknown concentration from a calibration curve, the change in transmittancy produced by adding a known amount of copper to the unknown was measured. The unknown concentration, x, was then calculated from Beer's law expressed as follows:

$$T_x = T_{a+x}^{x/(a+x)}$$

or

$$x = \frac{a \log T_x}{\log \dfrac{T_{a+x}}{T_x}}$$

in which T_x is the transmittancy of an aliquot of the sample, and T_{a+x} is the transmittancy of a similar aliquot containing a small added quantity of copper, *a*. The same principle has been used elsewhere, for example, in spectrographic analysis (*164*).

1·16 External means. Addition of the interfering constituent to the standards to compensate for their presence in the sample is illustrated in the following examples. Brabson, Harvey, Maxwell, and Schaeffer, in their work on the determination of silica in aluminum compounds, found aluminum to interfere (*49*). In their recommended procedure, aluminum is added to all standards, and samples of two different weights are used, the amount depending upon the silica content. It is necessary, therefore, to use a separate calibration curve depending on the sample weight.

Nichols and Rogers found it necessary to add iron to all samples and standards in the determination of molybdenum in plants by the thiocyanate method (*355*). Since maximum interference occurred in the presence of 2 mg. of iron, this amount was added to all samples.

Similarly, Schechter and Haller, in the determination of 1-chloro-2,4-dinitrobenzene in 2,4-dinitroanisole, compensated for interference due to the main constituent by adding it to all the standards (*430*). Their method, based on the Vongerichten reaction, involves reaction of the halogen compound with pyridine and alkali to give a violet color.

When interference is caused by some undesired absorptive material in the final solution, it is sometimes possible to discharge the main color by suitable means and then measure separately the color (or turbidity) causing the interference. The corrected value is then found by difference. This procedure is equivalent to adding the interfering constituents to the blank solution.

1·17 Use of monochromatic or filtered radiant energy. When interference results from absorption by some undesired constituent, the effect may be eliminated by use of the proper monochromatic radiant energy, provided that the two constituents present have their absorption bands in separate regions of the spectrum. For colored systems, this usually means having different hues. The efficacy of this method depends upon such factors as relative intensities of the bands, their shapes, their proximity, and the degree of monochromaticity of the source of energy. Since the problem here is one of physics, and therefore of measurement, details of such applications are given in Chapters 7 and 8.

The only question of chemistry involved is whether, in a given case, different preparative treatment or different reagents would yield a system better suited for measurement. Study of individual papers will show variations of the problem. [See Center and MacIntosh (*76*), Rodden (*404*), Brown (*58*), Greenleaf (*197*), Kozelka and Kluchesky

(*270*), Silverthorn and Curtis (*466*), Weissler (*524*, *525*), Clausen (*90*), Knudson, Meloche, and Juday (*262*), Zscheile and Porter (*572*), and Zscheile (*574*).]

C·THE COLORED SYSTEM

Desired colorimetric characteristics may be considered from the viewpoint of the system to be measured and also from the viewpoint of selection of the reagent for production of the colored system in those cases where the system is not already colored as such. Obviously, many properties of the system will depend on choice of the reagent used. Selection of the reagent for a given determination is made on the basis of those properties which it possesses and those properties which it contributes to the colored system.

1·18 The ideal colored system. Whether the sample is colored itself or requires addition of a color-forming reagent, the solution intended for colorimetric measurement should possess at least the following properties:

1. Stability for sufficient time to permit accurate measurement. In visual comparison, stability is essential if the standards are to be valid throughout the work. Instability, which usually results in fading, is sometimes the result of air oxidation, photochemical decomposition, effects of acidity, temperature, solvent, or other conditions. In many cases, little can be done to improve stability. In the determination of vitamin A with antimony trichloride, for example, the color measurement must be completed in a matter of seconds. The problem of measuring instable systems is discussed in a later section of this chapter.

2. Intense color. Although intensity of the color is usually determined by the concentration of colored product, one can exercise control over it by altering the solvent, in many cases, and by selecting a reagent of suitable sensitivity. An intense color is desirable both for detecting small amounts and for making accurate measurements irrespective of the concentration. For high concentrations, one can dilute the sample as required, since the volumetric measurements are probably the most accurate ones in the entire procedure, with the possible exception of weighing the sample.

3. Freedom from effects of minor variations in *p*H, temperature, or other conditions. Many colored systems require careful control of acidity, usually achieved by buffering, and some require temperature control, not only during development of the color but also while the measurement is being made. This is particularly true of measurements in organic solvents having large expansion coefficients, although there

are systems which change in color intensity with temperature for other reasons, such as shifting of equilibria involving the colored product.

4. Maximum absorption well within the spectral region where the eye is sensitive, if visual measurement is used. This corresponds to a range of about 475 to 675 mμ.

5. Solubility of the colored product. Reactions yielding a colloidal system or suspension are difficult to control and are generally susceptible to effects of other constituents, particularly electrolytes. Stabilizing additives such as agar and gelatin are only partially satisfactory. If the product is insoluble, it can sometimes be extracted and its color measured in another solvent, as in the determination of nickel (II) with dimethylglyoxime or copper with sodium diethyldithiocarbamate. Unless the extraction serves some useful purpose such as separation of the metal from interfering ions, as in the dithizone methods, this step is an undesirable complication and invites such errors as loss and contamination.

6. Conformity of the colored system to Beer's law. This is not essential, but it simplifies calibration and calculation of the results, especially in multicomponent analyses (see Chapter 7). Usually, if Beer's law is valid, one feels assured that satisfactory conditions are established for the concentration range in question. Conformity to Beer's law also permits use of variable-depth cells, as in the Duboscq-type comparators and in certain visual spectrophotometers.

1·19 The ideal reagent. Before considering the types of compounds used as color-forming reagents, we may note the general requirements which they should fulfill. Few, if any, of those now in use approach the ideal of meeting all these requirements, particularly freedom from interference. Knowing the requirements, however, the wise analyst is in a position to select from the reagents available the one best adapted to his purpose. The ideal colorimetric reagent, whether organic or inorganic, may be said to possess the following properties:

1. Stability in solution. Some reagents deteriorate in a few hours, and others ferment or develop mold growth on storage. Instability of the reagent usually requires frequent preparation of solutions and recalibration of the instrument for each new batch of reagent. Preservatives such as phenyl mercuric nitrate or ethanol are sometimes added.

2. Rapid color development. Aside from the standpoint of convenience, the effects of reaction time and temperature, which usually complicate slow reactions, are avoided if the color reaction is rapid and complete. An example of the interaction of time and temperature on a slow color reaction is encountered in the determination of furfural

with aniline acetate (*272*). As shown in Fig. 1·1, increased temperature accelerates both the color development and subsequent fading, the effect being more pronounced at low furfural concentrations. Temperature control at 25°C. and a reaction period of 20 minutes afford a suitable compromise between stability and time required to complete a determination.

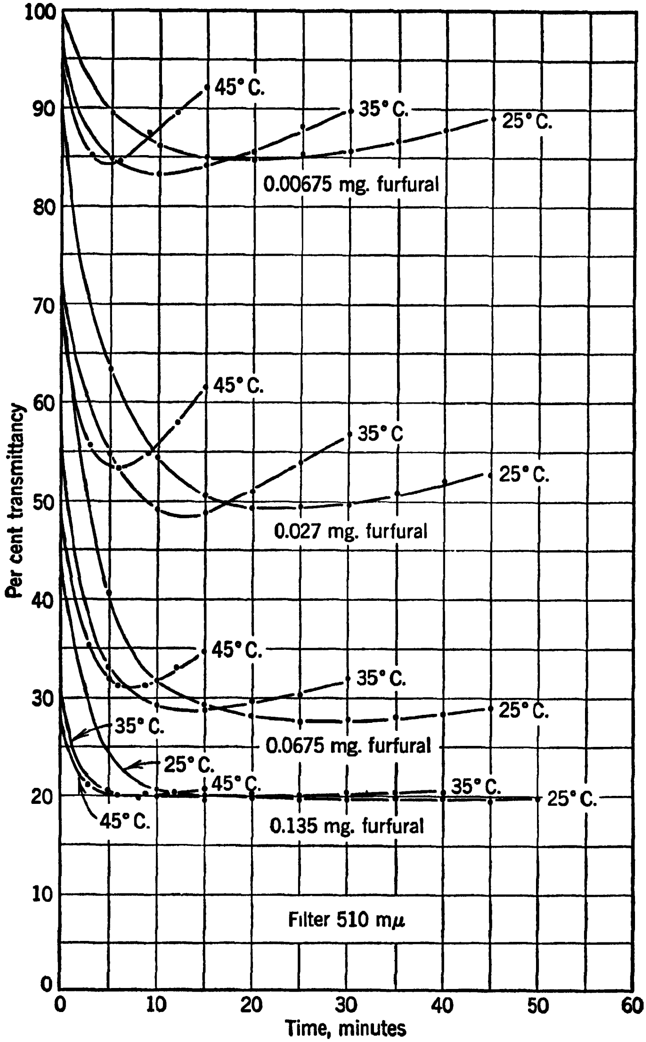

FIG. 1·1. Effect of time and temperature on aniline acetate method for furfural. (Krieger and Moss, unpublished data.)

3. Stoichiometric reaction with the desired constituent. If the reaction goes to completion, the exact amount of reagent added, if colorless, is not critical, and an excess may be used to allow for a wide range of concentrations and possible interference by side reactions which consume the reagent. The color intensity will then be proportional to the concentration, assuming that the colored product itself obeys Beer's law.

In the thiocyanate method for iron, the equilibrium $3NH_4SCN + FeCl_3 \rightleftharpoons Fe(SCN)_3 + 3NH_4Cl$ is affected by an excess of either of the reactants. Ammonium chloride, and other salts as well, reverse the reaction, making it easily subject to interference requiring careful control of conditions. Several colored ions, such as $Fe(SCN)^{++}$, $Fe(SCN)_2^{+}$, and $Fe(SCN)_6^{\equiv}$, are believed to be present and in equilibrium with one another.

4. Transparency, at least in the spectral region involved in the measurement. Colored reagents have to be measured out accurately whether or not the color reaction is stoichiometric, since the over-all color is dependent on the concentration of both the reagent and the desired constituent. In addition, color in the reagent detracts from the sensitivity of the method by causing high blank readings.

5. Selectivity or specificity, so that the color is a measure of the desired constituent only.

6. Freedom from interference by other constituents which might convert the reagent or desired constituent to an unreactive form or complex leading to incomplete color development.

7. Capacity to function in the all-round best solvent. The choice of solvent in which to make a color measurement is sometimes an important consideration. Metal thiocyanates are frequently measured in organic solvents for reasons of improved stability, sensitivity, or selectivity, or as a means of accomplishing a separation to avoid interference by other constituents. For example, Woods showed the iron-thiocyanate reaction to be approximately twice as sensitive in 60 per cent acetone as in water (*551*). Winsor used methyl Cellosolve (2-methoxyethanol) for the same purpose and demonstrated the advantage of working in a solvent of low dielectric constant, so as to prevent dissociation of the colored complex which would result in decreased sensitivity (*544*). Morehead also used methyl Cellosolve to stabilize the color in determining carbon disulfide in air by converting it to the diethyldithiocarbamate followed by measurement of the copper complex (*336*).

The reagent should be soluble in the solvent used for the sample, in order to avoid precipitation or separation of phases on mixing the two systems. Water-soluble reagents are ordinarily preferable.

It seems unlikely that the best solvent system has been found for every type of colored system, especially in view of the variety of new compounds now available. Possible improvements may be found in stability of the color, smaller instability constants, different wavelength minima for the absorption band, higher sensitivity, and other properties.

1·20 Methods used for developing color. Colorimetric determinations may be made directly on solids, liquids, or gases, if the desired constituent is in a form suitable for measurement and no interfering constituents are present. Ordinarily, the sample must be dissolved and subjected to certain preliminary treatment before measurement of the color. The ideal method would be sufficiently sensitive and selective to be directly applicable to a solution of the sample without involved preliminary treatment, although chemical reactions are generally required in producing the color for measurement.

Very useful compilations of chemical procedures for developing colored systems are available in the treatises of Allport (*3*), Lange (*282*), Snell and Snell (*473*), and Yoe (*558*). The best critical discussion of colorimetric methods for metals is the recent book of Sandell (*414*). General information regarding the use of organic compounds as analytical reagents may be found in the works of Feigl (*149, 150*), Flagg (*158*), Hopkin and Williams (*229*), Mellan (*316*), von Stein (*511*), Welcher (*527*), and Yoe and Sarver (*567*), including applications to colorimetry. Diehl (*123*), Feigl (*151*), Fischer and coworkers (*154, 155, 156*), Haendler and Geyer (*206*), Prodinger (*387*), and Sarver (*427*) have also helped to systematize and develop the general theory of reactions between metals and organic reagents.

The third volume of Snell and Snell (*473*) is devoted to methods for organic constituents and contains procedures for many compounds of biological interest. The treatise of Peters and Van Slyke (*380*) and the laboratory manuals of Hoffman (*225*) and of Karr, Reinhold, and Chornock (*251*) are devoted entirely to clinical methods, many of which involve colorimetry. A useful bibliography of colorimetric methods for inorganic ions has been prepared by Stillman and published by the American Society for Testing Materials, along with various other papers presented at the 1944 Symposium on Analytical Colorimetry and Photometry (*5, 488*).

The outline in Fig. 1·2 designates the most important types of colorimetric methods according to the chemical reactions used and the products obtained. With it one should be helped, not only in keeping in mind what has been found to work, but also in improvising possible new methods. It differs from Mellon's classification (*318*) in not listing separately the methods for organic and inorganic constituents or dis-

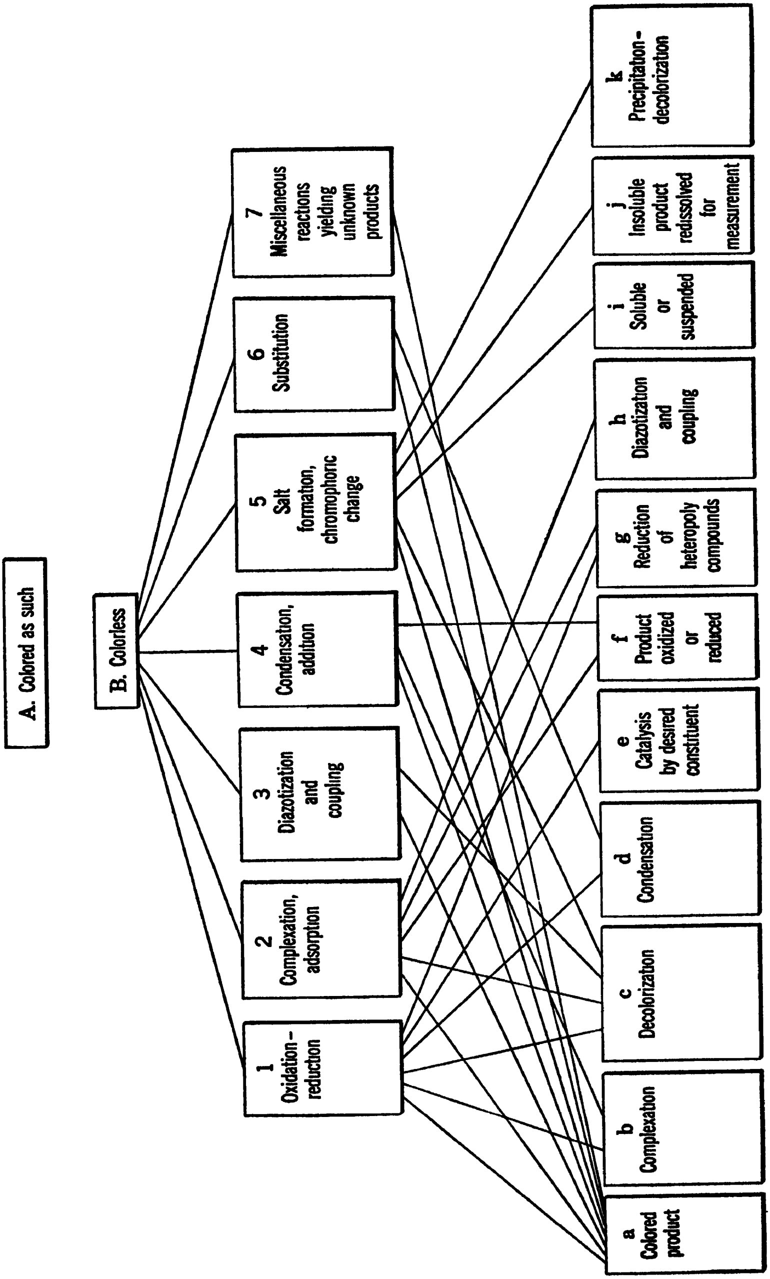
A. Colored as such
B. Colorless
1 Oxidation-reduction
2 Complexation, adsorption
3 Diazotization and coupling
4 Condensation, addition
5 Salt formation, chromophoric change
6 Substitution
7 Miscellaneous reactions yielding unknown products
a Colored product
b Complexation
c Decolorization
d Condensation
e Catalysis by desired constituent
f Product oxidized or reduced
g Reduction of heteropoly compounds
h Diazotization and coupling
i Soluble or suspended
j Insoluble product redissolved for measurement
k Precipitation-decolorization

FIG. 1·2. Classification of colorimetric methods according to the reactions used in developing the color.

tinguishing between methods for elements, compounds, or ions. Methods for constituents already colored, and requiring no color development, are assigned to Class A. Class B includes those methods in which a color reaction must be carried out, the desired constituents being colorless.

1·21 Systems colored as such. Except for certain kinds of organic compounds, few constituents are sufficiently absorptive within the limits of the visible spectrum for direct measurement of low concentrations, although many may be determined directly by their absorption in the ultraviolet or infrared regions. Hemoglobin in blood and bilirubin in serum, for example, can be determined by measurement of their colors or comparison with suitable standards. Chlorophylls and carotenes are also sufficiently colored for direct measurement.

Praseodymium, neodymium, and samarium can be determined in the presence of other rare earth elements, such as lanthanum, cerium, and gadolinium, by direct colorimetric measurement (*404*). Rare earth salts exhibit complex absorption spectra, some having as many as seven distinct bands in the visible. With the spectrophotometer, each band can be measured separately without the use of color-forming reagents.

The color of nitrogen dioxide has been utilized in a method for its determination in air with a photoelectric filter photometer (*211*).

1·22 Colorless systems. The colorless constituents are determined by developing colors with certain reagents or by decolorizing suitable colored systems. The following examples show how typical methods for determining colorless constituents fall logically into several groups according to the reactions on which the methods are based and the types of products obtained.

In a large number of methods, more than one reaction is involved, and several may proceed simultaneously with development of the color. For example, in the molybdenum blue method for phosphorus, the complex molybdiphosphate is first formed and then reduced to molybdenum blue. The classification is thus B2g, representing the two essential steps in the process. In determining ethanol with Schiff's reagent, the first step is oxidation to the aldehyde, which is then condensed with the reagent to form the colored product, the classification being B1d.

Many compounds for which a single, specific reaction is not available are determined by transforming them to compounds for which there are suitable methods. These are classified according to the final reaction involved in the color development. The determination of saccharin by Nesslerizing the ammonia formed on hydrolysis is listed under complexation reactions, since the hydrolysis step is part of the preliminary treatment and unrelated to the color reaction. A method even more indirect is used for calcium. Calcium is precipitated as the oxalate, which is then dissolved and oxidized with a measured amount of ceric sulfate. The excess ceric ion is measured in terms of the amount of iodine liberated from potassium iodide (*448*). The first step would come under the heading B5j, and the final step is of the Type B1a, since iodide is oxidized to the colored product I_2.

An indirect method for rhenium is based on the catalytic reduction of sodium tellurate by chlorostannous acid. The amount of tellurium formed is a measure of the desired constituent. A protective colloid is added to keep the tellurium in the colloidal state for photometric measurement. As little as 10^{-6} mg. of rhenium can be measured by this method (*384*), which is of the Type B1c.

An example of Class B1g, in which the oxidation-reduction step is followed by reduction of a heteropoly compound to form the desired color, is the determination of small amounts of reducing sugars with alkaline copper salts. The cuprous oxide formed is dissolved and measured in terms of the amount of molybdenum blue formed on reaction with molybdiphosphoric acid.

Reactions used quantitatively without exact knowledge as to the nature of the colored product or the reaction used are assigned to Class B7a. They may be entirely sound methods, the chief qualification being reproducibility and specificity under the conditions used.

Other examples of the various types of methods outlined in Fig. 1·2 are readily apparent. A representative list of typical methods taken from recent publications is given in Tables 1·7 and 1·8. Because of the large number and importance of methods based on oxidation-reduction, diazotization and coupling, and complexation, it seems of interest to discuss each of these topics specifically.

1·23 Oxidation-reduction. In methods involving oxidation of the desired constituent the product may be colored and suitable for measurement directly. Frequently, further reactions, such as complexation with a metal, condensation, or decolorization, are carried out before the final color measurement is made. Methods involving oxidation and reduction of the desired constituent are combined under the same heading, since, in many cases, the same chemical reaction serves as a basis for determining both oxidizing and reducing agents. This avoids designating a reaction as an oxidation in one case and a reduction in another. An example of an oxidation-reduction method with formation of a colored product directly would be the periodate method for manganese.

A method for benzoic acid based on oxidation to salicylic acid followed by formation of the reddish-purple ferric complex is an example of the B1b class.

Numerous methods of the B1c type are in use, such as the determination of ascorbic acid based on its decolorizing action on methylene blue.

Malic acid is oxidized with permanganate and the product condensed with dinitrophenylhydrazine. This method comes under Class B1d.

Reduction of heteropoly compounds. The name *heteropoly* was proposed by Rosenheim (*406*) to designate the class of complex inorganic compounds of which ammonium molybdiphosphate is the most familiar member. Many of these compounds are used in a wide variety of analytical procedures which have been reviewed recently by Wright (*553*). Typical heteropoly compounds are listed in Table 1·3. The formulas for the heteropoly acids are those proposed by Rosenheim. Later work with x-rays indicates different structures, such as that mentioned below for molybdisilicic acid.

TABLE 1·3 HETEROPOLY ACIDS

Central Atom	*Valence*	*Parent Acid*	*Heteropoly Acid*
B	3	$H_9(BO_6)$	$H_9[B(Mo_2O_7)_6]$
Si	4	$H_8(SiO_6)$	$H_8[Si(Mo_2O_7)_6]$
P	5	$H_7(PO_6)$	$H_7[P(Mo_2O_7)_6]$
Te	6	$H_6(TeO_6)$	$H_6[Te(MoO_4)_6]$
I	7	$H_5(IO_6)$	$H_5[I(MoO_4)_6]$

Coordinating groups around the central atom are usually oxides of molybdenum, tungsten, or vanadium. Variations occur in the ratio of coordinated groups to central atoms, the state of oxidation of the constituent atoms, and the coordinating groups themselves, as in the molybditungstiphosphates, which are examples of mixed compounds.

Heteropoly compounds, if colored, may be measured directly by colorimetric means. Otherwise, they are reduced to colored products. For example, with silicon there is formed the yellow molybdisilicic acid, $H_4[Si(Mo_3O_{10})_4]$, in which the desired constituent is the central atom. None of the reactants is colored. In the tungstivanadiphosphate method for vanadium, the desired constituent occupies part of the coordination sphere of the complex ion (*555*).

The reduction products are generally blue and are very intensely colored, which makes the methods highly sensitive to low concentrations. Metals forming insoluble 8-hydroxyquinolates may be determined indirectly, since the reagent precipitated with the metal will reduce an equivalent amount of a suitable heteropoly compound. In addition to inorganic constituents such as the central elements arsenic, germanium, phosphorus, and silicon, and the coordinating metals molybdenum, tungsten, and vanadium, numerous organic compounds are determinable by methods based on reduction of the heteropoly reagents. Some of these are listed in Table 1·4.

TABLE 1·4 METHODS BASED ON REDUCTION OF HETEROPOLY COMPOUNDS

Desired Constituent	*Reagent*	*References*
Albumin	Molybditungstiphosphoric acid	*473*
Arsenic	Ammonium molybdate	*80, 297, 552*
Carbon monoxide	Molybdisilicic acid	*454*
Cysteine	Tungstiphosphoric acid	*473*
Diethylstilbestrol	Molybditungstiphosphoric acid	*508*
Dihydroxyacetone	Molybdiphosphoric acid	*473*
Germanium	Ammonium molybdate	*44, 239*
Lactose	Molybdiphosphoric acid	*473*
Phenols	Molybditungstiphosphoric acid	*473*
Phosphate	Ammonium molybdate	*182, 259, 455, 552*
Protein	Molybditungstiphosphoric acid	*473*
Silicate	Ammonium molybdate	*44, 552*
Strychnine	Tungstiphosphoric acid	*473*
Tin	Molybdisilicic acid	*19*
Tryptophane	Molybditungstiphosphoric acid	*473*
Urea	Molybditungstiphosphoric acid	*473*
Vanillin	Molybditungstiphosphoric acid	*473*

1·24 Diazotization and coupling. A large number of sensitive methods based on coupling a diazonium salt with an aromatic nucleus are in use, particularly in clinical work. The desired constituent may be either diazotized or coupled, depending on the compound, and nitrous acid itself may be determined by the same reaction (*398*). Partial selectivity can be realized according to whether the coupling requires basic or acidic medium. For example, phenyl-β-naphthylamine can be determined in the presence of phenols by acid coupling with diazotized sulfanilamide (*350*). Phenol, which requires alkali coupling, cannot be determined directly in the presence of the amine by this method since both compounds couple under alkaline conditions.

Table 1·5 lists a number of compounds determined by diazotization methods. Metals forming insoluble 8-hydroxyquinolates may be determined indirectly by dissolving the precipitate and coupling the reagent with a diazonium salt. Urea is determined by allowing it to react with a measured amount of nitrous acid and determining the excess nitrous acid by diazotization.

TABLE 1·5 METHODS BASED ON DIAZOTIZATION AND COUPLING

Desired Constituent	*Reagent*	*References*
Aluminum	8-Hydroxyquinoline	*473*
p-Aminobenzoic acid	N-(1-naphthyl)-ethylenediamine	*132*
Bilirubin	Sulfanilic acid	*473*
Carnosine	Sulfanilic acid	*473*
Coumarin	*p*-Nitroaniline	*129*
Cresol	Sulfanilic acid	*473*
Ethylene chlorohydrin	Sulfanilic acid	*473*
p-Hydroxyphenylacetic acid	Sulfanilic acid	*473*
Nitric oxide	*m*-Phenylenediamine	*452*
Nitrite	Sulfanilic acid, α-Naphthylamine	*288*
Nitrite	Sulfanilamide, N-(1-naphthyl)-ethylenediamine	*254, 456*
Penicillin	Sodium nitrate, *p*-Aminobenzoic acid, N-(1-naphthyl)-ethylenediamine	*189*
Sulfonamides	Sodium nitrate, *p*-Aminobenzoic acid, N-(1-naphthyl)-ethylenediamine	*284*
Tyrosine, tyramine	Sulfanilic acid	*473*
Urea	Sulfanilic acid and phenol	*473*

1·25 Complexation. By a complex is meant here systems such as $Cu(NH_3)_4^{++}$, and $[P(W_3O_{10})_4]^{\equiv}$, representing ionic combinations, and nickel dimethylglyoxime, representing a molecular combination. The possibilities with such systems are very large, the objective in forming the systems being to render either an undesired constituent noninterfering or a desired constituent determinable.

Methods based upon measurement of colored complexes are most numerous in the field of metal determinations, and most of these methods utilize organic reagents for developing the color. Among the methods involving complexation with inorganic reagents are those for various nonmetals, including arsenic, carbon, phosphorus, and silicon, and the metals molybdenum, tungsten, and vanadium, usually based on formation of heteropoly complexes (except carbon); also included are the methods for metals measured as ammines (copper, nickel), chlorides (copper,* iron, platinum), iodides (bismuth, platinum) thiocyanates (iron, cobalt, nickel), and hydrogen peroxide complexes (titanium, uranium).

Some organic compounds are determined as metal complexes, for example, carbon disulfide (as copper diethyldithiocarbamate), biacetyl

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* The copper complex formed in concentrated hydrochloric acid has an intense absorption maximum at 970 mμ in the near infrared. By measuring this band one can avoid interference by iron, cobalt, and nickel, an attractive feature in metallurgical work *(180)*. Sensitivity is intermediate with respect to the ammonia and dithiocarbamate methods.

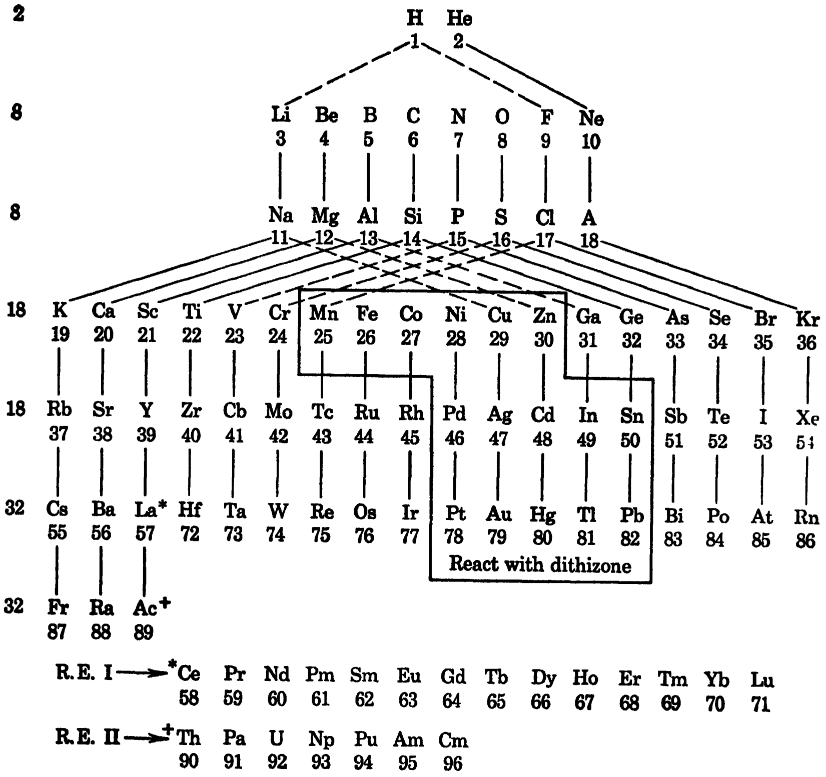
(as nickel dimethylglyoximate), and ethyl acetate (as ferric acetohydroxamate). Because of the importance of organic reagents in colorimetry and the wide interest in this subject, complexation can probably be best considered from the standpoint of organometallic complexes. Moreover, the general requirements of electronic structure and valence apply whether the coordinating group is organic or inorganic.

Of the three general types of metal complexes, only one is formed by chelation through ionic linkages. The other two require somewhat more exacting conditions as regards ring size and electronic configuration, since the coordinating atom (usually nitrogen, oxygen, or sulfur) must be suitably situated and the metal atom must be capable of accepting and sharing the electrons from the donor atom. It will be noted that the transition elements, having two incomplete shells, distinguish themselves particularly in forming covalent (coordination) complexes. The close relation among these elements is brought out in the Bohr form of the periodic table, Table 1·6, which places them together in one group. Many of them form colored complexes with dithizone. Among their similarities, aside from affinity toward organic reagents, are variable valence, paramagnetism, small radius, and tendency to form colored ions. The alkali and alkaline earth metals, forming ions with the same relatively compact electronic configuration as the inert gases, are colorless and practically unreactive toward organic reagents.

The number of unpaired electrons in the metal atom can usually be deduced by measuring the magnetic moment of the complex formed. The simple ions, having complete electron subgroups, are diamagnetic. Ions of the transition elements and rare earths are paramagnetic, their magnetic moments being determined by the number of unpaired electrons. In the iron atom, for example, the electronic structure is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6 4s^2$. Only the third shell is incomplete, ten $3d$ electrons (five pairs) being the usual number for the rare gases. Ferrous ion is formed by loss of the two $4s$ electrons, and ferric by loss of the two $4s$ electrons and one $3d$. With five $3d$ orbitals or grouplets available, the six $3d$ electrons of the ferrous ion must be distributed so as to form one pair and four unpaired electrons, since the tendency is to avoid pairing. In the ferric ion, with five $3d$ electrons, all five are unpaired and the magnetic moment is correspondingly higher.

In the ionic bonds, which are nondirectional, the surrounding groups are held primarily by electrostatic forces, and their electrons do not complete the shells of the metal atom. Resonance between ionic and covalent forms does not exist, since resonating structures must possess the same number of unpaired electrons.

TABLE 1·6 MODIFIED BOHR FORM OF PERIODIC TABLE OF THE CHEMICAL ELEMENTS



2	H 1	He 2																
8	Li 3	Be 4	B 5	C 6	N 7	O 8	F 9	Ne 10										
8	Na 11	Mg 12	Al 13	Si 14	P 15	S 16	Cl 17	A 18										
18	K 19	Ca 20	Sc 21	Ti 22	V 23	Cr 24	Mn 25	Fe 26	Co 27	Ni 28	Cu 29	Zn 30	Ga 31	Ge 32	As 33	Se 34	Br 35	Kr 36
18	Rb 37	Sr 38	Y 39	Zr 40	Cb 41	Mo 42	Tc 43	Ru 44	Rh 45	Pd 46	Ag 47	Cd 48	In 49	Sn 50	Sb 51	Te 52	I 53	Xe 54
32	Cs 55	Ba 56	La* 57	Hf 72	Ta 73	W 74	Re 75	Os 76	Ir 77	Pt 78	Au 79	Hg 80	Tl 81	Pb 82	Bi 83	Po 84	At 85	Rn 86
32	Fr 87	Ra 88	Ac+ 89															

React with dithizone

R.E. I ⟶ *Ce 58, Pr 59, Nd 60, Pm 61, Sm 62, Eu 63, Gd 64, Tb 65, Dy 66, Ho 67, Er 68, Tm 69, Yb 70, Lu 71

R.E. II ⟶ +Th 90, Pa 91, U 92, Np 93, Pu 94, Am 95, Cm 96

Complexes of metals with coordination number six are considered stereochemically to be octahedral, since this configuration is usually the best basis for predicting the number of stereoisomers for a given compound. Octahedral structures are formed by certain metals having valences of 2, 3, 4, and 5, including platinum, palladium, iron, copper, nickel, cadmium, zinc, cobalt, chromium, rhenium, iridium, manganese, ruthenium, aluminum, titanium, silicon, and arsenic. Many of these, including copper, platinum, palladium, iron, cobalt, and nickel, also form complexes with coordination number four. Two stereochemical configurations occur corresponding to coordination number four: (1) tetrahedral, and (2) planar, or square. The following relation exists between the electronic structure of the valence bonds and the spatial configuration of the complex, assuming that, in addition to *d* electrons, only the two *s* and six *p* electrons of the next shell are involved in the bonds (*374*). If the complex is tetrahedral, *s* and *p* electrons only are involved (sp^3 bonds). If *d* electrons are also involved (dsp^2 bonds), the complex is planar and *cis-trans* isomerism is possible. Among the planar complexes are the glyoximes, dithiooxalate, ethyldithiocarbamate, and tetracyanide ion of nickel, palladium, and platinum cyanides and thiocyanates, and palladium dimethylglyoxime (*374*). Nickel ammines, hydrazines, and acetylacetonates are tetrahedral. Tetrahedral complexes are paramagnetic, and the planar ones diamagnetic. Magnetic measurements of complex compounds are of considerable interest in solving problems related to structure, and there is an extensive literature on the subject. The recent monograph of Selwood on magnetochemistry gives a thorough coverage of the work in this field and suggests many interesting problems (*446*).

1·26 Organic reagents for metals. While the earliest use of organic reagents appears to be Pliny's testing for iron with nutgall tannins (*357*), modern developments in this field began in 1905 with Chugaev's work on dimethylglyoxime as a precipitant for nickel. Since then, hundreds of compounds have been proposed and studied, resulting in many excellent analytical procedures. Most of the reagents are used as precipitants for the metals, although many form soluble compounds which can be measured colorimetrically. Since certain of the metals exhibit more pronounced coordinating tendencies than others, it is not surprising that a wide selection of suitable reagents is available for some metals whereas others are devoid of a single reagent that can be considered satisfactory.

The theory of organic reagents is not highly developed, although considerable empirical work has been done in studying the available compounds and in correlating their chemical behavior with respect to structure and functional groups. Several atomic groupings have been found to be specific for certain metals, for example, the 1,2-dioxime configuration for nickel (*122*) and the cyclic N—C—C—N group for ferrous iron (*318*). Others are known and have been discussed by various writers, notably Feigl (*150*) and Prodinger (*387*). The effects of substituents on a reagent are usually unpredictable, although they often produce interesting and desirable properties, particularly as regards solubility. Yoe and coworkers (*559*) have made a systematic study of the reactions of hundreds of organic compounds with seventy-odd ions under various selected conditions. A number of useful reagents have been discovered as a result.

The organometallic complexes are usually characterized by intense color (often quite different from that of the ordinary metallic salts), low solubility in water, comparatively high solubility in organic solvents, definite composition, and extraordinary stability. Certain metal acetylacetonates are said to withstand distillation at temperatures above 300°C. (*123*), and copper phthalocyanine is reported to sublime undecomposed at 580°C. These are striking examples of the effects obtainable through complexation of the metals with suitable reagents.

Although not all the best reagents form cyclic structures, the greatest opportunities for developing sensitive methods lie in this direction rather than with the reagents forming the usual complexes such as heteropoly ions, thiocyanates, and metal ammines. The rings containing the metal ions are subject to the same rules as any ring structure regarding bond angles and other spatial considerations. As would be expected, the five- and six-membered rings predominate, although a few with four are encountered and others exist with seven or more rendered strainless by lying in more than one plane. Nondirectional ionic bonds generally do not exist once the ring is formed, as the metal atoms are held firmly by essentially covalent forces and the resulting products lack the ordinary saltlike properties.

Following are the more common of the functional groups which react with the metals:

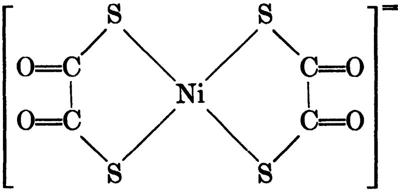
Acidic Groups	*Coordinating Groups*
$-\mathrm{OH}$	$=\mathrm{NOH}$
$-\mathrm{SH}$	$=\mathrm{O}$
$-\mathrm{CO_2H}$	$=\mathrm{N}-$ (cyclic)
$=\mathrm{NOH}$	$-\mathrm{NH_2}$
$-\mathrm{SO_3H}$	$-\mathrm{S}-$
	$=\mathrm{NH}$

Electrovalent bonds involve acidic groups, while coordinate bonds are formed through sharing of electron pairs furnished by the coordinating groups. Usually, there is little difference after ring closure occurs, when the bonds become predominantly covalent.

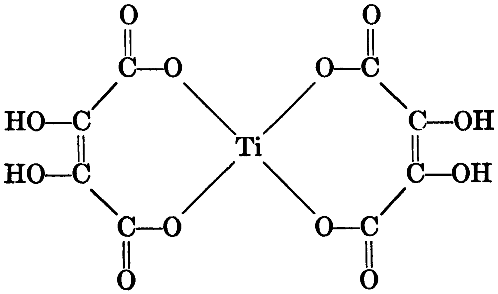
The general rules of organic precipitants for the metals apply also to the color-forming reagents, since, in many cases, the same chemical reaction serves for both precipitation-gravimetric and colorimetric methods. For colorimetric purposes, however, it is desirable that the colored product be soluble, so that no special treatment such as extraction or stabilization is required. The reactions usually involve chelation and, for a given reagent, may result in formation of a colored complex ion, lake, precipitate, or nonelectrolyte, depending on the metal and the conditions employed. Formation of chelate structures may take place through (I) acidic groups, (II) coordinating groups, or (III) both acidic and coordinating groups.

1·27 Compounds containing acidic groups. Complexes with reagents possessing acidic functional groups are usually formed by replacement of acidic hydrogens with the metal to form salts or ions. This type of reaction is not as common as the others, and the compounds formed are not as stable as other types. Inorganic acids may coordinate in two positions forming rings, usually of four members. With organic reagents, the rings may be four-, five-, or six-membered.

Potassium dithiooxalate, proposed as a reagent for nickel by Yoe and Wirsing (*568*), forms a magenta or purple complex ion in which the metal replaces the acidic hydrogens. X-ray studies of the complex have shown it to be coplanar and isomorphous with the palladium and platinum compounds (*374*). Beer's law is valid in the range 0.1 to 10 p.p.m., and maximum absorption occurs at 500 mμ. Silver gives a soluble red complex, and colored products are formed also with antimony, bismuth, copper, cobalt, and other ions, including ferrous and ferric iron.



Dihydroxymaleic acid gives a soluble compound with titanium. This reaction has been used by Mellor (*320*) in determining titanium and vanadium simultaneously by combining it with the well-known peroxide method, which can be used to measure both metals. The structure of the titanium complex is reported to be as follows:

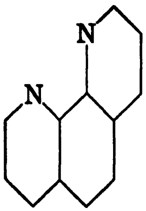


There is some uncertainty as to the function of the hydroxyl groups in the reaction. Being only weakly acidic, they may not be involved.

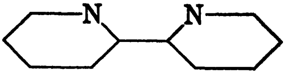
Many lakes and dyes having several acidic groups might be classified in this section, although the products formed with the metals are probably much more complicated than the two foregoing structures. Various reactions, such as salt formation, ring closure with coordinating groups, and formation of adsorption complexes of variable composition between the dyes and the heavy metal hydrous oxides, are possible. Reagents in this class include alizarin, quinalizarin, morin, aluminon, tannin, and titan yellow. Since the colored products of these reagents are usually insoluble, it is necessary to add protective agents to prevent precipitation. For example, in the hematoxylin method for aluminum, glycerin or "starch glycerite," prepared by heating starch and glycerin together, is used (*6*, *491*).

1·28 Compounds containing coordinating groups. Reagents which form metallic complexes through coordinate linkages contain nonmetal donor atoms such as oxygen, sulfur, or nitrogen. The bond is established by an electron pair furnished by the nonmetal atom, one pair for each coordination position of the metal. In such reagents, the organic molecule functions as a neutral constituent and forms a complex ion having the same charge as the metal ion itself, or a multiple thereof if a polynuclear product is formed (*123*).

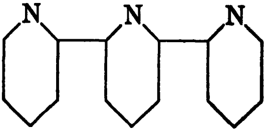
One of the most interesting of the atomic groupings showing selective action toward the metals is the cyclic N—C—C—N group, which occurs in 1,10-phenanthroline and related compounds. These reagents, in addition to forming a number of intensely colored compounds with copper, cobalt, and molybdenum, are practically specific in their reaction with ferrous iron, forming a hexammine ion. Among the reagents in this class are 1,10-phenanthroline, 2,2′-bipyridine, and 2,2′,2″-terpyridine. There is no indication that the most useful member of this class has yet been developed.



1,10-Phenanthroline



2,2′-Bipyridine



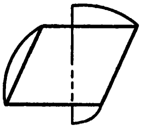
2,2′,2″-Terpyridine

Reactions of a number of 2,2′-bipyridine derivatives with ferrous iron have been reported by Burstall (*64*). Substituents in the 3 position cause a marked decline in the coordinating power of the compounds, with the result that they give only a feeble color with iron. 5-Bromo-2,2′-bipyridine gives an intense color. Disubstituted compounds with the corresponding positions in both rings occupied give no color with iron except the 3,3′-dicarboxylic acid. According to the earlier work of Blau (*39*), the acid does not give a color with iron. Burstall attributed the inactivity of the substituted bipyridines to a screening effect of the large groups near the nitrogen atoms. Measurement of dipole moments indicate that the rings are oriented with the nitrogen atoms on opposite sides of the bond between the rings.

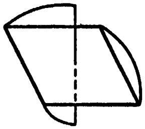
2,2′,2″-Terpyridine acts as a tridentate group and forms many stable and characteristic coordination compounds which may be classified in two series according to the molecular proportions of base to each atom of metal (*339*). In the monotridentate series, (M tripy X) and (M tripy X)X, occur the salts of copper, univalent and bivalent silver, zinc, cadmium, mercury, palladium, and platinum. Tervalent iridium chloride yields ($IrCl_3$ tripy). The second series of the bistridentate type is represented by (M 2 tripy)X_2 and (M 2 tripy)X_3-$n$$H_2O$, in which *M* is bivalent iron, cobalt, nickel, ruthenium, osmium, or tervalent cobalt, nickel, or chromium.

The nature of the ferrous complexes is fairly well understood, the ions being octahedral in structure. In bipyridine and phenanthroline, the ratio of reagent to metal is three to one. Twelve electrons are involved, one pair from each of six nitrogens. Since ferrous ion has the configuration $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6$, the complex would be represented by $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^2 4p^6$. Having no unpaired spins, it is, as would be expected, diamagnetic. Magnetic properties of these compounds have been reported by Cambi and Cagnasso (*71*).

The octahedral complexes of bipyridine and phenanthroline have the configurations shown in structures I and II. The two possible forms have been resolved (*528*).

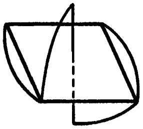


I

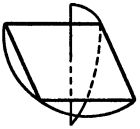


II

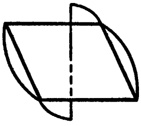
With terpyridine, three forms may be written, represented by structures III, IV, and V. Of these possibilities, IV is the most probable, since it



III



IV



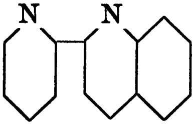
V

requires the least distortion of the triamine. The structure represented by III is questionable because it can exist in optically active forms and resolution among the terpyridine salts has not been achieved. Arrangement V remains as a possibility.

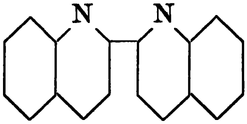
Bipyridine and phenanthroline offer several advantages over other reagents used for iron, such as stability of the color (one year with bipyridine) and wide latitude in *p*H variations (3 to 9). In addition, Beer's law is valid. The color reactions are stoichiometric and unaffected by excess reagent. Both reagents are about equally satisfactory.

None of the phenanthroline derivatives studied offers any advantage over the parent compound as a colorimetric reagent, although there are minor differences in sensitivity and hue (*349*). 2,2′-Bipiperidine, like bipyridine, is reported to form a colored ferrous complex (*39*).

Smirnoff found that the compounds represented by structures VI and VII do not react with iron and believed that the coordinating tendency of the nitrogen atoms was taken up by the double bonds in the adjacent rings (*469*). The biquinoline (VII) has been used as a reagent for copper (*52*). Smirnoff predicted that the three possible, unknown biisoquinolines would also react with iron.

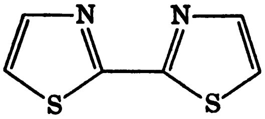


VI

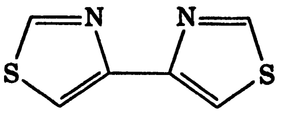


VII

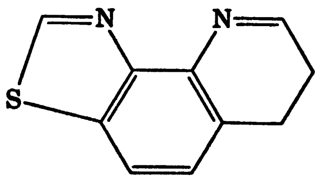
Erlenmeyer and Schmid studied the sulfur compounds VIII, IX, and X from the standpoint of bond angles in the N—C—C—N group (*144*). Distortion of these bonds was believed to be responsible for the varied behavior of the compounds toward iron. The compound VIII



VIII
2,2′-Bithiazoline



IX
4,4′-Bithiazoline



X
Quinthiazole

gives a color with ferrous iron only if heated, and the color disappears on cooling. No color with iron was obtained with IX. Quinthiazole gives a yellow color with 1 p.p.m. of ferrous iron.

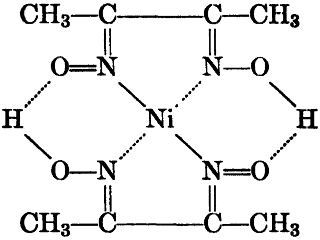
Although phenanthroline, bipyridine, and terpyridine have the same reactive atomic grouping and react similarly with iron, there are several significant differences. First is the difference in wavelength minima for the absorption bands, the respective values being 508, 522, and 552 mμ. This provides for some flexibility in avoiding an interfering color. Next comes some difference in the reactivities. Only terpyridine reacts with cobalt to give a color sufficiently intense for colorimetric work. The copper-ammonia complex, if reduced with hydroxylamine in the presence of phenanthroline, gives an insoluble purple cuprous complex. On addition of a water-soluble organic solvent, the cuprous salt dissolves, undergoing a striking change in hue from purple to reddish amber. The colored product is not extractable with solvents. Bipyridine gives a less intensely colored product. Terpyridine does not react. A method for copper based on this reaction has been reported (*348*). Another interesting reaction, unpredicted from previous knowledge, is the formation of a highly colored product on reduction of molybdate with chlorostannous acid in the presence of phenanthroline or bipyridine. The reaction will detect molybdenum, even in the presence of iron, but the complex is not sufficiently stable for quantitative purposes (*349*). Such variations in behavior as these are only isolated examples of the many possibilities offered by organic reagents.

1·29 Compounds containing acidic and coordinating groups. Reagents in this class form metal complexes through replacement of hydrogen by the metal accompanied by ring closure to form "inner complexes," usually nonelectrolytes. The compounds formed are ordinarily insoluble in water, soluble in nonpolar organic solvents, unionized, highly colored, and remarkably stable. The replaceable hydrogen may be attached to (1) nitrogen, (2) sulfur, or (3) oxygen.

1·30 Nitrogen-containing compounds. Dimethylglyoxime is probably the best known of the organic precipitants for metals, having been in use for over forty years. It is also a valuable colorimetric reagent for nickel and may be used in several ways. The insoluble nickelous salt may be produced in the form of a suspension in aqueous medium and extracted with a solvent, in which it is then measured colorimetrically. A second method is to form the more soluble nickel (IV) complex by oxidation with hypochlorite, bromine water, or lead dioxide, and measure its color in aqueous solution. For purposes of separation, it is possible to extract the precipitate with chloroform and transfer the nickel back to aqueous solution by shaking the chloroform extract with dilute acid, the reagent remaining in the chloroform.

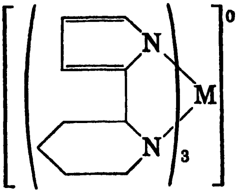
Other 1,2-dioximes, such as α-furildioxime and 1,2-cyclohexanedionedioxime, react similarly.

The nickel (II) salt of dimethylglyoxime is diamagnetic and has the following planar structure:



Dimethylglyoxime may also be used as a color reagent for copper and ferrous iron. The subject of dioximes as analytical reagents, along with numerous procedures, has been reviewed recently by Diehl (*122*). An interesting item of chemistry is the stabilization of several iron-dioxime complexes with sodium dithionite. Pyridine has served similarly with diethylaminobutanedionedioxime (*199*).

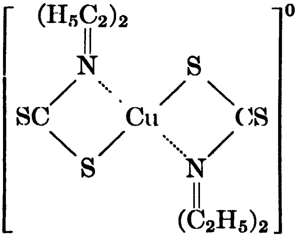
In α-pyridyl pyrrole it is possible to replace hydrogen on the pyrrole nitrogen and obtain an inner complex with the metal attached to both nitrogen atoms. This reaction does not occur in water, however. To obtain the complex the base is heated in naphthalene containing the powdered metal. Aluminum, cadmium, cobalt, copper, iron, nickel, and zinc react, giving typical inner complex salts. These are insoluble in water but readily soluble in benzene and other nonpolar solvents. The zinc and aluminum compounds are fluorescent. The iron salt is reddish purple with maximum absorption in the visible at 540 mμ. A stronger band occurs in the near ultraviolet. α-Pyridyl pyrrole has not been studied from the point of view, although it may be of interest in the determination of certain free metals, as the color reaction would not have to be preceded by the usual lengthy process of ashing or acid digestion to dissolve the metal. The reagent contains the cyclic N—C—C—N grouping, although it differs from phenanthroline and bipyridine in having a replaceable hydrogen attached to nitrogen. The type of complex is different, accordingly.



α-Pyridyl pyrrole complex

1·31 Sulfur-containing compounds. Sodium diethyldithiocarbamate is probably the most widely used reagent for small amounts of copper. Considerable work has been reported on this determination (*3*, *414*, *473*). The color reaction takes place in neutral or slightly ammoniacal solutions, the product being an insoluble, brown inner complex salt. In low concentrations, precipitation does not occur during the time required for the color measurement, but with larger amounts (about 1 p.p.m.) it is necessary to stabilize the suspension with a suitable colloid or to extract with a solvent such as chlorobenzene, amyl acetate, or carbon tetrachloride. Some fading occurs on extraction, along with a corresponding decrease in sensitivity except as the total volume of solution is decreased and the copper thereby concentrated. Iron appears to be the most serious of the interfering metal ions, but its effect can be substantially reduced by converting it to the unreactive citrate complex.

Maximum absorption of the copper complex varies slightly with the solvent but is close to 440 mμ. The structure is reported to be

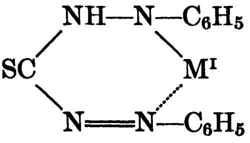


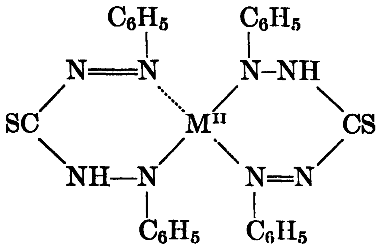
in which copper replaces a thiolhydrogen (the sodium salt is ordinarily used) and forms secondary valence bonds through the electron pairs furnished by nitrogen atoms (*316*).

Dithizone, diphenylthiocarbazone, also a sensitive copper reagent, is better known as a reagent for lead, mercury, zinc, and cadmium. After its introduction by H. Fischer in 1925, it has found extensive application not only as a color-forming reagent but also as a means for isolating small traces of a number of the metals. The metal dithizonates, being nonelectrolytes, are water insoluble but extractable with certain organic solvents, particularly chloroform and carbon tetrachloride. Although dithizone forms a relatively large number of different metal complexes, considerable selectivity can be attained by proper *p*H control during the extraction step, as the various metals form complexes varying widely in stability with respect to *p*H.

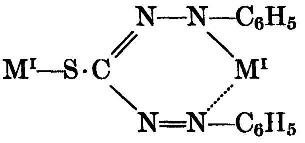
Dithizone gives dark green solutions in chloroform or carbon tetrachloride and dissolves in aqueous alkali to give a yellow color. The metal dithizonates are mostly reddish purple, although a few are yellow or brown. They are believed to exist in one of two forms, depending on whether the reagent reacts in the keto or enol form. Some metals form both kinds of complexes. Keto forms are the more common, the enol complexes forming only in alkaline solution or with an excess of the metal ion. The two types of complexes can be interchanged, in cases where enol forms exist, by varying the *p*H of the aqueous solution in contact with the dithizone solution. Formulas proposed for the dithizonates include the following:

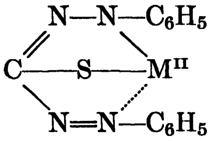
Keto





Enol





Keto complexes are formed by bismuth, cadmium, cobalt, copper, lead, mercury, palladium, silver, thallium, tin, and zinc. Many of these also form enol complexes. A complete review of the theory and applications of the dithizone technique, along with numerous recommended procedures, is given by Sandell (*414*). The effect of *p*H and other conditions on the extraction of metals with dithizone has been studied by Clifford and Wichman (*94*) and by Biefeld and Patrick (*37*). Fischer, in a recent paper (*155*), discusses the relations of *p*H stability, configuration, and ionic radius. Bivalent ions form the most stable complexes.

Since dithizone is readily oxidized, a reducing agent such as hydroxylamine is used to keep the reagent in the reduced form. Interference by other metals present is usually avoided by proper control of *p*H or by addition of a complexing reagent to convert the interfering metal to an unreactive complex ion of greater stability than the corresponding dithizonate. Cyanide, thiocyanate, tartrate, citrate, and thiosulfate are commonly used for this purpose.

After separation of the metal as the dithizonate, the metal complex may be decomposed by acid or alkali and the metal determined by any of the usual procedures. Alternatively, the metal may be measured by one of the following dithizone methods:

1. Removal of excess dithizone by extraction with ammonia and colorimetric measurement of the remaining metal dithizonate (one-color method).

2. Measurement of either the green color of the excess reagent or the color of the complex without separation (mixed-color method). Measurement of the complex gives somewhat greater sensitivity. Spectrophotometers are preferred.

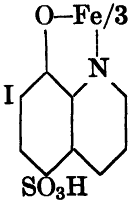
3. "Colorimetric" titration of a measured volume of dithizone solution with a standard solution of the metal being determined until a match with the sample is obtained. A continuous gradation in hue from green through gray to purple is obtained with lead, for example. The hues rather than the color intensities are matched. It is also possible to titrate the dithizone with bromine in carbon tetrachloride.

Errors in the dithizone methods result usually from contamination and incomplete separations. Contamination is not easily avoided, as dithizone is among the most sensitive reagents known.

Related to dithizone are di-β-naphthylthiocarbazone and di-(*p*-biphenyl)-thiocarbazone, the latter reagent being particularly sensitive to low concentrations of arsenicals (*502*).

1·32 Oxygen-containing compounds. 8-Hydroxyquinoline, better known as a precipitant for a number of metals such as aluminum, magnesium, iron, and zinc, is also used as a colorimetric reagent for ferric iron. It is highly sensitive and gives a bright green complex having maximum absorption at 440 mμ. An improved reagent, proposed by Yoe and Hall (*563*), is ferron, 7-iodo-8-hydroxyquinoline-5-sulfonic acid, which gives a similar green hue. The iodo and sulfonic acid substituents improve the stability of the color. The reagent is water soluble, giving a yellow solution. Other ions, including ferrous iron, do not give colors with ferron. Maximum sensitivity is obtained at *p*H 2.6, and careful control of *p*H is required. The color is stable for at least 9 days. A method for fluoride based on its bleaching effect on the ferron complex was described by Fahey (*148*). This reaction is an example of recomplexation, the colorless fluoferrate ion, $FeF_6^{\equiv}$, being more stable than the iron-ferron complex.

Three molecules of ferron react with the ferric ion to give the following structure, in which the metal is linked with oxygen and nitrogen in a five-membered ring:



1·33 Identification of the complexes. Methods used for determining the composition of the colored products may include chemical analysis of the isolated material, studies of electrode potentials, transference, x-ray and electron diffraction, optical activity, magnetic susceptibility, polarography, and direct color measurements. Spectrophotometric measurements on solutions are convenient and direct, since possible changes in the composition and properties of the material being examined, which might result from concentration or drying the solution, are avoided.

In the reaction between a metal M and a reagent R to form a colored complex according to the equation

$$x\mathrm{M} + y\mathrm{R} \rightarrow z\mathrm{C}$$

the ratio x/y can be determined by measuring the absorbancies for mixtures of varying proportions of metal and reagent. In an excess of metal ion, the maximum concentration of the complex obtainable from a concentration p of the reagent is pz/y. Similarly, with excess reagent, the maximum concentration obtainable from a concentration q of the metal is qz/x. If the measured absorbancies in the two cases are $(A_s)_p$ and $(A_s)_q$ and the molar absorbancy index a_m is the same, corresponding to a single colored compound, a_m is given by

$$a_m = \frac{(A_s)_p}{pz/y} = \frac{(A_s)_q}{qz/x}$$

or

$$\frac{y}{x} = \frac{p(A_s)_p}{q(A_s)_q}$$

Molland used this method (*333*) to identify the iron complex of 8-hydroxyquinoline-5-sulfonic acid as $(C_9H_6O_4NS)_3Fe$. The copper complex, studied by means of its interference with the iron reaction, was shown to be $Cu(C_9H_6O_4NS)_2Cu$. The copper complex is presumably planar and diamagnetic, and the iron complex octahedral and paramagnetic.

Tarbell and Bunnett, in studying the reaction between di-(*p*-biphenyl)-thiocarbazone (DBT) and certain arsenicals, plotted absorbancy against the ratio arsenical/DBT. The ratio at which absorbancy became constant indicated the composition (*502*).

Disodium-1,2-dihydroxybenzene-3,5-disulfonate, proposed by Yoe and Jones as a reagent for iron, forms two types of ferric complexes, red in alkaline medium and blue in acid (*565*). At *p*H 9.5, the color intensity for a given amount of iron increases with reagent concentration until the mole ratio of reagent to iron is 3:1. Thereafter, no increase in intensity occurs, the reaction being stoichiometric, as indicated in Fig. 1·3. At *p*H 4.0, the color increases indefinitely with an increase of reagent, and the composition of the complex is not apparent. In acid solution, it is therefore necessary to control reagent concentration closely in order that the color be a measure of the iron concentration only. Measurement of the color in alkaline medium is recommended for this reason and because of greater sensitivity, 1 part in 200,000,000 for the red complex and 1 in 30,000,000 for the blue complex, when viewed in Nessler tubes.

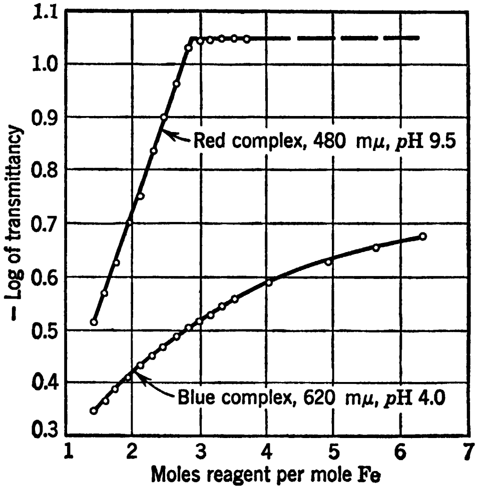


FIG. 1·3. Identification of iron complexes with disodium-1,2-dihydroxybenzene-3,5-disulfonate. [Yoe and Jones, *Ind. Eng. Chem., Anal. Ed.*, **16**, 111 (1944).]

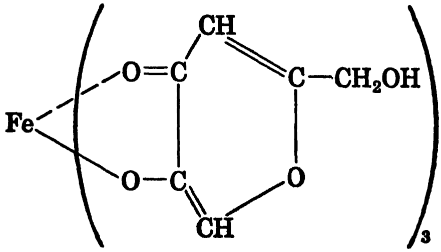
A simple and rapid spectrophotometric method of "continuous variations" for identifying complex ions in solution has been described by Vosburgh and Cooper (*512*). Based on the method of Job (*244*), it has been applied to chromate-dichromate mixtures in addition to complex ions of nickel with 1,10-phenanthroline and with ethylenediamine, and also to the copper-ammonia ions, in which case the components react in more than a single ratio.

For reactions of the type

$$A + nB \rightleftharpoons AB_n$$

in which the complex AB_n is formed from the ions A and B, solutions of A and B in the same concentration are mixed in varying proportions. The proportion giving maximum absorption indicates the composition of the complex and the value of n, if only one product is formed. If more than one product is formed, measurements at various wavelengths give different results. The theory has been developed for as many as three colored ions.

With this method, the ferric complex of kojic acid, 2-hydroxymethyl-5-hydroxy-γ-pyrone, was found to contain a 3:1 ratio of kojic acid to iron (*344*). The complex probably has the following form:



in which iron replaces hydrogen and the three bidentate groups occupy the six coordination positions of the metal. The hydroxymethyl group is not involved in the reaction. Kojic acid is the only pyrone derivative used as a color-forming reagent, and iron is the only metal with which it gives a color. It lacks the sensitivity and freedom from interferences of such reagents as phenanthroline and bipyridine.

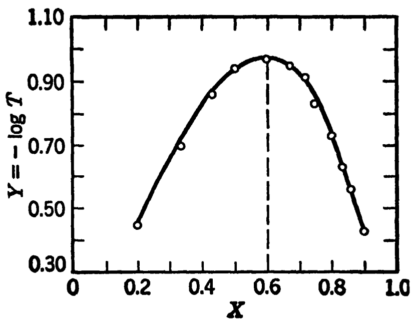


Fig. 1·4. Identification of iron complex of 4-hydroxybiphenyl-3-carboxylic acid. [Yoe and Harvey, *J. Am. Chem. Soc.*, **70**, 648 (1948).]

Yoe and Harvey applied the method of continuous variations to the ferric complex of 4-hydroxybiphenyl-3-carboxylic acid (*564*). Although only one type of complex was observed, considerable dissociation was indicated by the increase in color intensity with reagent concentration. This dissociation was found to occur at reagent-iron ratios as high as 10:1.

Figure 1·4 shows the effect of continuous variations in reagent-iron concentrations on the color intensity. The ordinate Y is the observed absorbancy, corrected for the color of the iron and of the reagent, and is equal to the difference between the observed absorbancy and the absorbancy calculated assuming no reaction. Since the iron and reagent solutions are practically colorless at the concentration used (1.79×10^{-4} M for each), Y is equal to the observed absorbancy

($-\log T$). X, a measure of the relative amounts of iron and reagent, is equal to the liters of 1.79×10^{-4} M reagent solution added to $(1 - X)$ liters of ferric iron solution of the same concentration. The curve passes through a maximum at $x = 0.6$, and the ratio of iron to reagent is thus 2:3. Measurements were made at 575 mμ. Similar work at other wavelengths gave curves of the same shape, indicating no other complexes to be present.

Several structures have been assigned to the ferric thiocyanate complex in aqueous solution, including the familiar $Fe(SCN)_6^{\equiv}$ (*434, 435*) and, more recently, anions such as $Fe(SCN)_2^{+}$ and $Fe(SCN)^{++}$ (*33, 191, 334*). Evidence for the complex cation reported by Bent and French was based on a spectrophotometric study of the reaction

$$m\mathrm{Fe}^{+++} + n\mathrm{SCN}^{-} \rightleftharpoons \mathrm{Fe}_m(\mathrm{SCN})_n$$

Values of m and n were determined by preparing two series of iron-thiocyanate solutions, one of which varied in iron concentration and the other in thiocyanate concentration. Slopes of the straight lines relating log absorbancy with log ion concentration gave the number of ions involved in the complex. In both cases values of one were obtained, indicating an ion of the formula $Fe(SCN)^{++}$. This conclusion was substantiated by migration experiments conducted in a direct-current field. Several later investigators believe the composition of the complex depends upon the proportions of the reactants.

The thiocyanate complex of molybdenum was studied by Hiskey and Meloche (*222*), who showed that the metal has a valence of five and that thiocyanate and molybdenum react in a ratio of 3:1. The product forms slowly and is extractable from aqueous solution with ether.

A sensitive method for aluminum is based on formation of a lake with eriochromcyanine-R, an orange-red dye. The nature of the complex formed was studied by Millner (*327*) and found to depend on the relative proportions of aluminum and reagent. In an excess of reagent, the aluminum and reagent react in a molecular proportion of 1:3. This was shown by measurements of absorbancy on solutions containing a measured amount of aluminum and increasing amounts of the dye. The absorbancy increases uniformly with an increase in reagent concentration as more of the complex forms up to the point where an equivalent amount is added. Beyond that point, the absorbancy increases at the same rate as in the absence of aluminum, because of the color of the dye itself. The intersection of the two linear portions of the curve relating absorbancy and reagent concentration occurs at a point corresponding to the 1:3 ratio.

The situation is somewhat different if varying amounts of aluminum are added to a fixed concentration of reagent. In a curve relating absorbancy and aluminum concentration for these solutions, there are indications of four linear portions in the curve, as shown in Fig. 1·5. These four parts of the curves have been interpreted as follows:

The first, representing an aluminum-reagent ratio of 1:3, rises sharply since the reagent is in excess. A less steep portion follows, corresponding to a ratio of 1:2, and finally a third, still less steep, corresponding to a ratio of 1:1. The curve then flattens out with no further increase in absorbancy. The first product formed is the highest in stability and color intensity, the second, intermediate, and the third, the least stable and least highly colored. Since all three forms are in equilibrium, it is not surprising that conditions such as acidity and concentration of various reagents must be carefully controlled.

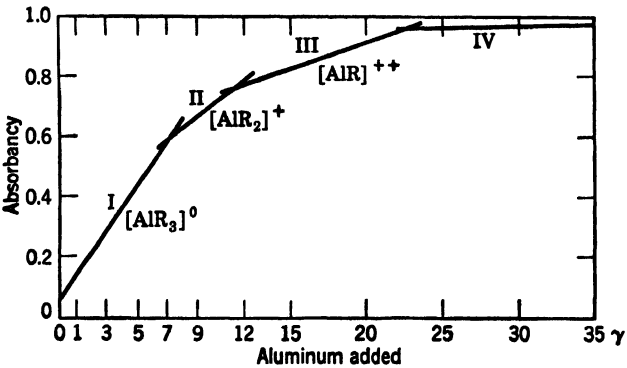


FIG. 1·5. Effect of aluminum concentration on the composition of eriochromcyanine-R-complexes. [Millner, *Z. anal. Chem.*, **113**, 83 (1938).]

1·34 Measurement of instable systems. One of the most desirable features in a colorimetric method is a colored product sufficiently stable for accurate measurement. If the color is to be compared visually with standards, either the colored product must be stable or the standards have to be frequently prepared. An alternative is to use a series of artificial "permanent" standards, such as colored glasses or solutions intended to match the color given by the desired constituent. Ordinarily, one avoids using a color reaction yielding an instable product as the basis of a quantitative method. If the color is not very stable, the measurement should be made by photoelectric means with careful timing of the reaction. Ingenious methods of dealing with instable systems have been devised, and several such methods will be described.

When confronted with a problem in stability, one usually alters the conditions in an effort to stabilize the system. A knowledge of the reactions involved in the fading or other change in color is helpful. Use of solvents having low dielectric constants was mentioned in Section 1·19 as a means of improving the stability of certain complexes capable of dissociating.

Instability of the colored system may manifest itself in a variety of ways, such as: (1) fading beginning immediately after adding the color-forming reagent, (2) fading at some time after obtaining maximum color, (3) fading and color development proceeding concurrently, or (4) intensification of the color on standing by slow development of either the desired color or some other color (or turbidity) associated with decomposition of the desired color.

Where the color is changing rapidly, the simplest expedient is to time the reaction and allow exactly the same interval between addition of the reagent and measurement of the color for all samples and standards. Another method is to take several readings at measured intervals and extrapolate to zero time for the initial value. Duke recommends this method in determining alcohols with ammonium hexanitratocerate (*126*). A single 2-minute reading is also satisfactory (*436*). Accurate timing is also recommended in the determination of aluminum with hematoxylin (*6*, *272*) and in the determination of furfural with aniline acetate, although, in these cases, slow development of the color is more of a consideration than is instability of the color when developed. In any method where timing is a critical factor, one should be alert to the possible interaction of temperature, concentration, and time as in the aforementioned determination of furfural. (See Fig. 1·1.)

Probably the most fugitive color used on a large scale is the blue color obtained in the Carr-Price reaction between antimony trichloride and vitamin A. Here it is imperative to time the reaction and color measurement as carefully as possible, usually taking the reading between 5 and 30 seconds after adding the reagent, procedures varying among laboratories. Efforts have been made to stabilize the colored system by adding certain reagents such as acids, dehydrating agents, and phenols, without apparent success (*170*, *407*).

A difficult problem involving instability and interference is the determination of nicotinamide (niacin amide) in pharmaceutical products containing nicotinic acid (niacin) and other materials. Aniline and cyanogen bromide are used to develop the color by the König dianil reaction. This reaction is not specific for the amide, since the acid also gives the color and with greater intensity. Because of their different rates of color development and fading, however, it is possible to determine both constituents simultaneously in a mixture. Maximum color development is attained in 6 minutes with the acid and in 3 to 4 minutes with the amide.

Several variations in developing the color have been proposed. Lamb recommends adding the aniline before the cyanogen bromide, since a delay of a few minutes in the addition of aniline following the cyanogen bromide introduces a large error, whereas a 15-minute delay has no effect if the aniline is added first (*280*). On the other hand, the reaction with the amide is 50 per cent more sensitive if the cyanogen bromide is added first, followed in 10 minutes by addition of the aniline reagent. Melnick and Oser recommend adding the reagents in rapid sequence, since the color intensity given by nicotinic acid is practically twice that given by the amide in spite of a 30 to 35 per cent reduction in sensitivity in measurement of the acid (*321*). Observations are taken periodically during the color development, since maximum values are used and fading occurs rapidly once the maximum has been reached.

The following readings are taken: (1) blank on reagents against water, (2) the sample, and (3) the sample plus known amounts of the constituent being determined. The last two readings are made against an equal amount of the sample, containing aniline but no cyanogen bromide, to correct for color in the sample. Lamb recommends plotting the absorbancies and extrapolating to obtain the value for the sample, using the three readings lying on a straight line, according to Beer's law. A filter photometer with 420-mμ filter was used for this determination in both laboratories.

A method for the determination of ascorbic acid is based on measurement of the decolorization of 2,6-dichlorophenolindophenol (*224*). Readings are taken at 5 and 10 seconds while reduction of the dye, a second-order reaction, is proceeding. Obviously, the problem of mixing the reagent and sample, placing the solution in the photometer, and taking the readings in the allotted time requires careful planning by the analyst. By way of simplification, the authors recommend a direct-reading photometer (not the null type), a special pipet delivering exactly 5 ml. of reagent in 1 second, and a nomograph for converting absorbancies to ascorbic acid concentration. By taking readings at 5 and 10 seconds, interference from reducing agents present in the sample is shown to be largely eliminated. In this method the dye used as the reagent is diluted so as to give an absorbancy of 0.398 (transmittancy of 40 per cent) so as to obtain maximum photometric sensitivity.

An analysis by Brew and Scott (*53*) of certain factors affecting the determination of vitamin A with antimony trichloride should be applicable to other instable systems as well. Materials interfering with this determination are grouped into three classes:

1. Materials giving an intense color, similar to that of the vitamin, which fades on standing (M).
2. Materials which give a color of low intensity, uniformly increasing (N).
3. Materials which are colored or produce a color relatively constant with respect to time (O).

On the basis of this classification, the method provides for dealing mathematically with color instability and interference by these materials. Several readings are taken as illustrated in Fig. 1·6. The first reading is taken immediately after reaction (reading A), a second at 5 minutes (reading B), and a third at 10 minutes (reading C). In addition, a measured amount of the vitamin is added to a second portion of the sample and run as an "internal standard" (reading D) and a reading of the diluted sample containing no reagent is made at 440 mμ to correct for carotenoids or other colored constituents present. A constant temperature at 30°C. is used on all measurements involving the antimony trichloride.

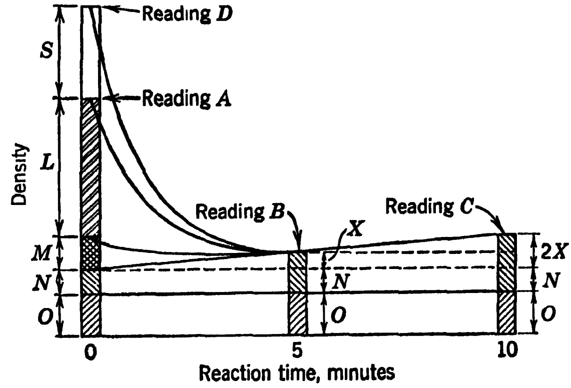


Fig. 1·6. Derivation of formula for calculation of vitamin A. [Brew and Scott, *Ind. Eng. Chem., Anal. Ed.*, **18**, 47 (1946).]

The absorption reading A is equal to the total absorption of the vitamin (1) and any absorption due to the three types of interference listed, represented by quantities M, N, and O. Because of fading, the contributions of L and M are zero at the end of 5 minutes, when the B reading is taken, at which time O is constant and N has increased by an increment X. At the end of 10 minutes, represented by reading C, the quantity N has increased by an additional increment X, making its value $N + 2X$. These relations may be expressed as follows:

$$A = L + M + N + O$$

$$B = X + N + O$$

$$C = 2X + N + O$$

and combined to give $L = A - 2B + C - M$. The value M is obtained from reading E, which measures the carotenoid color at 440 mμ. This is used in correcting for the contribution of carotenoids to the color obtained with antimony trichloride at 620 mμ, according to the relation:

$$M = KE$$

or

$$L = A - 2B + C - KE$$

The value of K is determined by a separate calibration with known materials.

Internal compensation is used in eliminating possible interference from any materials inhibiting the color reaction between the vitamin and the antimony trichloride as measured by the absorption value L. A measured increment of the vitamin is added to a portion of the samples, and reading D taken. This includes the absorbancies comprising reading A and, in addition, the absorbancy contributed by the known amount of vitamin added. The absorption equivalent to the increment added is equal to $D - A$. For 10 units of vitamin added, according to the recommended procedure,

$$\frac{\text{Units of vitamin A}}{10} = \frac{L}{D - A}$$

or

$$\text{Units of vitamin A} = \frac{10(A - 2B + C - KE)}{D - A}$$

This general method of dealing with an instable system should be of value also in other applications.

A novel approach to the problem of measuring instable systems, aside from the chemical, mathematical, and graphical methods mentioned, is the "dynamic" method of Gibson and Taylor (*179*). Their apparatus permits measurements on flowing solutions, so that the time of observation with respect to the time of mixing can be accurately controlled. Observations are unaffected by the transient nature of an instable colored system, the technique having been developed for vitamin A determinations.

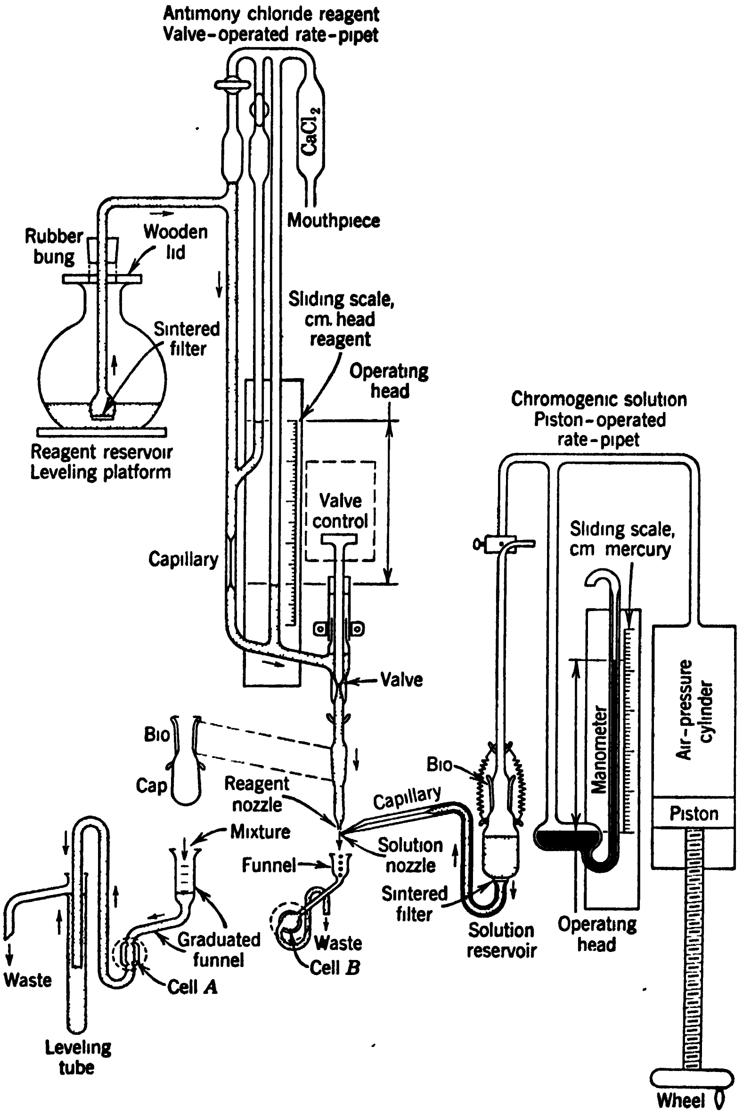


FIG. 1·7. Diagram of apparatus for dynamic study of vitamin A reaction. [Gibson and Taylor, *Analyst*, **70**, 449 (1945).]

The antimony chloride reagent and sample solution flow from separate reservoirs under pressure. (See Fig. 1·7.) Thorough mixing occurs when the two solutions issue from their respective capillary jets. Immediately after mixing, the colored solution passes through the absorption cell in which its color is measured at any desired reaction time.

A stream of the mixture can be regarded as a series of thin strata or increments, each at a definite stage of color development. Two types of cells are used, according to whether the increments or strata are to be viewed edgewise in passing or viewed parallel to the direction of flow. In the latter case the increments constitute a series of superposed layers, the observed absorption then being the sum of the absorptions for all the increments.

By this method it is possible to study time factors and other variables in detail so as to select the most favorable conditions for measuring the color. For research purposes it should be of considerable value in isolating certain effects occurring simultaneously and resulting in a colored system too instable for measurement by conventional means.

1·35 Other photometric methods. Photometric methods outside the scope of this chapter include turbidimetry, nephelometry, and fluorimetry. To a considerable extent, the chemical principles underlying these methods apply also in colorimetry, particularly as to chemical processes involving preparation of the sample and removal of undesired constituents. Use of these other photometric methods is much less extensive than that of colorimetry, although they are important branches of the general subject of photometric analysis.

Turbidimetry is based on measurement of the turbidity produced by causing the desired constituent to precipitate out in the form of finely divided particles of suitable dimensions. Ordinarily the suspension is viewed so that transmitted light is measured. There is some overlapping between colorimetry and turbidimetry, as many colorimetric methods involve measurement of colored materials in suspension which would precipitate readily at higher concentrations. Turbidimetric measurements are best made with blue light, which gives the highest photometric sensitivity. Sensitivity is favored by an insoluble product that is opaque or highly colored.

Nephelometry is similar to turbidimetry, the chief difference being that the suspension is viewed at right angles to the light beam. Turbidimetric measurements can be made visually with Nessler tubes or other simple apparatus by looking directly through the suspension at a source of diffuse light. Photometers measuring transmitted light are also widely used. For nephelometric determinations, however, the light beam itself is not measured. Instead, the measurement is made on the light scattered at right angles. This geometric arrangement is reported to be more sensitive in measuring low concentrations because of the dark background representing zero concentration in contrast to full illumination at zero concentration in turbidimetric measurements. The same chemical principles for producing the turbid systems are used in both turbidimetry and nephelometry. The theory and practice are discussed in detail in Yoe's treatise on nephelometry (*558*). Photoelectric instruments for measuring scattered light and fluorescence at right angles to the illuminating beam are now commercially available.

Fluorimetry includes those procedures based on determining a constituent by measuring its fluorescence or that produced by adding suitable reagents. Many fluorimetric methods are capable of high sensitivity, although these are usually only "semiquantitative." One of the notable exceptions is the determination of boron with benzoin (*533*). At a concentration of 2 to 10 μg. in 50 ml. of solution, boron can be measured with an average error of only 1.5 per cent. Excellent results were obtained on steel samples containing boron in the range of 0.001 per cent.

Fluorimetric procedures for vitamin determinations are used on a large scale, particularly for riboflavin and thiamin. Although considerable preliminary treatment is required in isolating the vitamin from the food products in which it occurs before measurement, the fluorimetric method has proved to be invaluable (*99*). The general subject of fluorimetric methods has been reviewed by White (*531a*), and the theory discussed by Sandell (*414*).

A few turbidimetric, nephelometric, and fluorimetric methods are listed in Tables 1·7 and 1·8, along with the colorimetric methods. These are assigned, respectively, the letters T, N, and F under the heading designating the types of methods.

TABLE 1·7 METHODS FOR INORGANIC CONSTITUENTS

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
Aluminum	Water	Hematoxylin	B2a	*6, 262, 491*
	Biological materials	Alizarin Red S	B2a	*86*
	Aluminum steel, leach liquors	Aluminon	B2a	*106, 361*
	...	8-Hydroxyquinoline	B2a	*174, 332*
	...	Morin	F	*532*
	Steel, bronze, minerals	Pontachrome Blue-Black R	F	*526*
Ammonia	Latex, saline soil solutions	Nessler's reagent	B2a	*165, 395*
	Soil, plant extracts	Graves's reagent	B2a	*549*
Antimony	Biological materials	Rhodamine B	B2a	*168, 169, 301*
	Copper-base alloys	Iodide, hypophosphite	B2a	*227*
	Biological materials	Iodide	B2a	*304*
Arsenic	Biological materials	Molybdate	B2g	*44, 80, 235, 242, 297, 300, 421, 494*
Barium	....	Chromate	B5j	*167*
Beryllium	Ores	Quinizarin	F	*159*
	Rocks	Morin	F	*420*

TABLE 1·7 METHODS FOR INORGANIC CONSTITUENTS (*Continued*)

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
Bismuth	....	8-Hydroxyquinoline	B2a	*332*
	Lead, biological materials, aluminum alloys	Iodide	B2a	*178, 249, 359, 484, 534*
	Biological materials, copper	Dithizone	B2a	*233, 557*
Boron	Soils, plants, steel	Quinalizarin	B2a	*34, 521*
	Soils, plants	Curcumin	B2a	*353*
	Steel	Benzoin	F	*533*
Bromide	.. ..	Rosaniline	B6a	*509*
Cadmium	Biological materials	Di-β-naphthylthiocarbazone	B2a	*83*
	Rocks	Dithizone	B2a	*419*
Calcium	Animal tissues	Oxalate, iodate-iodide	B1a	*117*
	Soil, plant extracts	Oxalate	T	*547*
	Blood serum	Oxalate, ceric sulfate, iodide	B5j	*448*
	Water, foods	Oxalate, permanganate	B1c	*441*
	Water	Potassium oleate	T	*413*
Carbonate	Sodium bicarbonate	*p*-Nitrosothymol	B5a	*495*
Carbon dioxide	Gases	Methyl Red	B5a	*545*
	Gases	Phenolphthalein	B5c	*478*
Carbon monoxide	Gases	Palladium chloride	B1a	*447*
	Gases	Molybdisilicic acid	B2g	*454*
	Gases	Beef blood, pyrogallic and tannic acids	B2a	*100*
Carbon disulfide	Gases	Diethylamine, copper acetate	B2a	*336*
Chlorate	Caustic soda	*o*-Tolidine	B1a	*540*
Chlorine	Water	*p*-Aminodimethylaniline	B1a	*66*
Chromic oxide	Air mists	Diphenylcarbazide	B1a	*135*
Chromium	Steel	Persulfate	B1a	*466*
	Steel	Perchloric acid	B1a	*468*
	Silicate rocks	Diphenylcarbazide	B1a	*408, 415*
Cobalt	Biological materials, silicate rocks, ores, stainless steel	Thiocyanate	B2a	*29, 418, 569, 389*
	Plant tissue, metallurgical products	Nitroso-R salt	B2a	*369, 458, 538, 570*
	....	Oxalate, lead dioxide	B1b	*73*
	....	Ammonia, ferricyanide	B2a	*115*
	... .	α-Nitroso-β-naphthol	B2a	*515*
	Biological materials	Nitrosocresol	B2a	*138*
	....	8-Hydroxyquinoline	B2a	*332*
	....	Terpyridine	B2a	*347*
	....	β-Nitroso-α-naphthol	B2a	*561*
	... .	*o*-Nitrosoresorcinol	B2a	*367*

TABLE 1·7 METHODS FOR INORGANIC CONSTITUENTS (*Continued*)

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
Copper	Latex	Dimethyldithiocarbamate	B2a	*165*
	Ferrous metals	....	A	*180*
	Distilled liquors, plants, phosphorus, aluminum alloys, wort, yeast, beer, dyes, intermediates, rubber	Diethyldithiocarbamate	B2a	*176, 369, 51, 373, 489, 368*
	....	Tetraethylenepentamine	B2a	*109*
	....	Rubeanic acid	B2a	*76*
	Ammonium hydroxide, steels	Ammonia	B2a	*60, 328*
	Nickel-plating solutions, foods, metals, silicate rocks	Dithizone	B2a	*58, 342, 31, 416*
	....	Biquinoline	B2a	*52*
	.	8-Hydroxyquinoline	B2a	*332*
	. .	1,10-Phenanthroline	B2a	*348*
	...	Triethanolamine	B2a	*562*
Cyanide	Biological materials	Ferrous sulfate	B2a	*171, 177*
	....	Chloramine T, pyridine, pyrazolone	B4a	*141*
Fluoride	Rocks, minerals, water	Ferron-iron complex	B2c	*148*
	Water	Zirconium-alizarin	B2c	*278, 279*
	Water	Thorium-alizarin	B2c	*500*
	Minerals	Calcium chloride	N	*486*
	Glass	Peroxytitanic acid	B2c	*371*
Fluosilicic acid	Hydrofluoric acid	Molybdate	B2a	*68*
Gallium	Silicate rocks	8-Hydroxyquinoline	F	*424*
Germanium	....	Molybdate	B2a	*257*
	Silicate rocks	Molybdate	B2g	*44, 239*
Gold	....	Chlorostannous acid	B1a	*153*
Hydrogen peroxide	Air	Titanic sulfate	B1a	*136*
Hydrogen sulfide	Gases	Bismuth, cadmium, uranium salts	B5i	*152*
Indium	....	8-Hydroxyquinoline	B2a	*331*
Iodine	Blood	Ceric sulfate, arsenic acid	B1e	*79*
Iron	Water, foods, aluminum alloys, brass, biological materials, ores	1,10-Phenanthroline and derivatives	B2a	*7, 25, 376, 186, 236, 163, 428, 314, 349*
	Ores, malt beverages, biological materials, distilled liquors, pharmaceuticals	2,2′-Bipyridine	B2a	*314, 240, 176, 437, 306, 346*
	Sea water, engine oils, foods	Thiocyanate	B2a	*391, 396, 503, 551, 544, 59*
	Water, minerals, ceramics, plating solutions	Disodium-1,2-dihydroxybenzene-3,5-disulfonate	B2a	*565, 196*

TABLE 1·7 METHODS FOR INORGANIC CONSTITUENTS (*Continued*)

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
Iron (*continued*)	. . . .	Thioglycolic acid	B1b	*498*
	. . . .	Ferron and 8-hydroxyquinoline	B2a	*89, 497, 563, 332*
	Ores	Salicylic acid	B2a	*308, 309*
	. . . .	Salicylaldoxime	B2a	*231*
	. . . .	Dioximes	B2a	*199*
	Water	*o*-Nitrosophenol	B2a	*108*
	. . . .	Nitroso-R salt	B2a	*198*
	. . . .	4-Hydroxybiphenyl-3-carboxylic acid	B2a	*564*
Lead	Silicate rocks, steel, biological materials, sugar, maple syrup, cosmetics, pharmaceuticals	Dithizone	B2a	*416, 56, 21, 85, 232, 271, 531, 194, 377, 438, 22, 23, 270, 203, 474*
	. . . .	Diphenylcarbazide	B1a	*286, 287*
Lead, red	Paint pigments	Acetic acid	B5i	*499*
Magnesium	Plants	8-Hydroxyquinoline	B2a	*460*
	Plants	8-Hydroxyquinoline, molybdate	B5j	*369*
	Alloys, plants, soils, liquors	8-Hydroxyquinoline, ferric chloride	B2j	*520, 175, 119*
	Soils	Phosphate, molybdate, chlorostannous acid (or aminonaphtholsulfonic acid)	B5j	*395, 253*
	Soils, fertilizers	Titan yellow	B2a	*181, 292*
Manganese	Steel, biological material, caustic soda, rubber, dyes, intermediates	Periodate	B1a	*310, 352, 369, 539, 368, 335*
	Caustic soda	Iodate	B1a	*101*
	Steel	Persulfate	B1a	*466*
	Plants, soils	Formaldoxime	B2a	*459, 461*
Mercury	Biological material	Dithizone	B2a	*269*
	Biological material	Di-β-naphthylthiocarbazone	B2a	*82, 234*
		Diphenylcarbazide	B2a	*276*
Molybdenum	Rocks, plants, soils, steel, ores	Thiocyanate	B2a	*355, 369, 415, 250, 202*
Neodymium	Rare earth mixtures	. . . .	A	*404*
Nickel	Bronze, steel, silicate rocks	Dimethylglyoxime	B2a	*443, 208, 298, 3, 418, 372, 330*
	Steel, ores	Ammonia	B2a	*15, 312*
	Foods, biological materials	Diethyldithiocarbamate	B2a	*2*
		Dithiooxalate	B2a	*568*
Nitrate	Nitration spent acid	Ferrous sulfate	B1b	*140*
	Soils, plants, water	Brucine	B6a	*358, 549*
	Sodium nitrite	Nitrobenzene, acetone	B6d	*444*
	Soils	Devarda's alloy, Nessler's reagent	B2a	*395*
Nitric oxide	Coke oven gas	*m*-Phenylenediamine	B3a	*452*
	Air	Sulfanilic acid, α-naphthylamine	B3a	*288*

TABLE 1·7 METHODS FOR INORGANIC CONSTITUENTS (*Continued*)

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
Nitrite	Water	Sulfanilic acid, α-naphthylamine	B3a	*398, 288*
	Water, foods	Sulfanilamide, N-(1-naphthyl)-ethylenediamine	B3a	*254, 456*
	Soils, plants	Dimethylaniline	B6a	*549*
Nitrogen	Plants, steel	Nessler's reagent	B2a	*253, 340, 30, 104*
	Soil, plants	Graves's reagent	B2a	*548, 550*
Nitrogen dioxide	Gases	....	A	*211*
Nitrosyl sulfuric acid	Nitration spent acids	Ferrous sulfate	B1b	*140*
Osmium	Iron	Thiourea	B2a	*422*
Oxygen	Gas	Ferrous thiocyanate	B1b	*453*
	Gas	Manganous chloride, iodide, starch	B1a	*543*
Phosphorus	Limestone, ores, steel, feeds	Molybdate, vanadate	B2a	*258, 50, 536, 77, 210, 352, 43*
	Water, soil, biological materials, plants, rubber, oils, steel	Molybdate, reducing agent	B2g	*182, 455, 483, 263, 552, 259, 369, 548, 253, 104, 26, 121, 133, 274, 160, 44, 185, 252, 462, 385*
	Water	Ferric thiocyanate	B2c	*546*
	Nonferrous metals	Ferric salicylate	B2c	*267*
Potassium	Water, biological materials, soils	Cobaltinitrite	B5j	*369, 458, 548, 402, 395, 463, 518*
	Biological materials	Dipicrylamine	B5k, B5j	*4, 104, 265*
Rhenium	Molybdenite, pyrolusite	Thiocyanate	B2a	*223, 238, 237*
	....	Tellurate, chlorostannous acid	B1e	*473, 384*
Selenium	Biological materials	Codeine sulfate	B7a	*188*
Silica, silicate, silicon	Water, steel, alumina, salts, alloys	Molybdate	B2a	*1, 261, 439, 403, 409, 74*
	Aluminum, magnesium, water	Molybdate	B2g	*44, 552, 49, 48, 492, 62, 248, 362*
Silicon tetrafluoride	Hydrocarbons	Molybdate	B2a	*69*
Silver	Photographic materials	Potassium tetracyanonickelate (II), dimethylglyoxime	B2a	*464*
	....	2-Thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine	B2a	*566*
Sodium	Biological fluids	Zinc uranyl acetate	B5j	*8, 112*
	. ...	Magnesium uranyl acetate	T	*289*
Sulfate	Biological materials	Barium chloride	T	*507*
	Biological materials	Benzidine	B5j	*260*

TABLE 1.7 METHODS FOR INORGANIC CONSTITUENTS (*Continued*)

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
Sulfur dioxide	Biological materials	Fuchsin-formaldehyde	B4a	*192*
Tin	Steel, alloys	Molybdisilicate	B2g	*19*
Titanium	Steel	Hydrogen peroxide	B1a	*465, 524, 525, 329*
	....	Tiron	B2a	*560*
	....	Hydrogen peroxide, dihydroxymaleic acid	B1a	*320*
Tungsten	Minerals	Thiocyanate	B2a	*201, 423*
	....	Titanous chloride, malachite green	B1e	*417*
Uranium	Thorium	Thiocyanate	B2a	*110*
Vanadium	Rocks, steel	Tungstiphosphoric acid	B2a	*415, 555*
	....	Hydrogen peroxide	B1a	*554*
Zinc	Biological materials, steel, plating solutions	Dithizone	B2a	*369, 216, 105, 57, 449*
	Biological materials	Di-β-naphthylthiocarbazone	B2a	*84*
	Soil, agronomic materials	5-Nitroquinaldic acid, chlorostannous acid	B2f	*291*
	Soil	Ferrocyanide	T	*41*
Zirconium	Steel	*p*-Dimethylaminoazophenylarsonic acid	B5j	*213*

TABLE 1·8 METHODS FOR ORGANIC CONSTITUENTS

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
Acetophenetidin (phenacetin)	Drugs	Chromic acid	B1a	*114*
Acetylene	Air, liquid oxygen	Copper salts	B2a	*173, 307*
Acids and esters	Tin plate, oils	Hydroxylamine, ferric chloride	B4b	*218, 220*
Acrolein	Beverages	Tryptophane	B4a	*87*
Alkyl benzene sulfonates	Surface-active agents	*o*-Tolidine, hypochlorite	B1a	*210*
Alcohols	Water	Ammonium hexanitratocerate	B2a	*126*
Alcohols (higher)	....	Benzaldehyde, sulfuric acid	B4a	*127*
Alcohols (higher)	Ethanol	Dimethylaminobenzaldehyde, other aldehydes	B4a	*95, 96, 375*
Aloe-emodin	Aloes	....	A	*490*
Aniline	....	Calcium hypochlorite	B1a	*145*
Amines (aryl)	....	....	...	*63, 139, 541*

TABLE 1·8 METHODS FOR ORGANIC CONSTITUENTS (*Continued*)

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
α-Amino nitrogen	Amino acids	Ninhydrin	B1d	*433*
Amino acids	Corn	Miscellaneous	....	*125*
p-Aminobenzoic acid	Blood	N-(1-naphthyl)-ethylenediamine	B3a	*132*
Ammonium compounds, quaternary	Textiles and paper	Potassium iodide	B2a	*207*
	Antiseptics	Bromophenol blue	B5a	*12, 13, 97*
Ammonium picrate	Waste water	.. ..	A	*412*
Amyl nitrite	Air	Phenol, copper sulfate	B7a	*230*
Anesthetic compounds	Drugs, biological materials	Nitrous acid, N-(1-naphthyl)-ethylenediamine	B3a	*24*
Arsenicals	Air	Di-(*p*-biphenyl)-thiocarbazone	B2a	*502*
Benzene	Air	Nitric acid, alkali	B6d	*124*
Blood	Packing-house scraps	Sodium dithionite	B1a	*394*
Caramel	Wines, vinegar, extracts	... .	A	*299*
Carboxymethyl cellulose (degree of substitution)	.. ..	2,7-Dihydroxynaphthalene	B4a	*147*
Chlorophylls, related materials	Plants	. . . .	A	*98, 200, 378*
	Plants	... .	F	*187*
Choline	Biological materials	Ammonium reinickate	B5j	*505*
Citral	Flavors, extracts	*m*-Phenylenediamine, chromic acid	B4a	*18*
Coumarin	Sweet clover	Diazotized *p*-nitroaniline	B3a	*129*
Crystal violet (leuco)	Assay	Benzoyl peroxide	B1a	*445*
Cyclopentadiene	C_5 petroleum fractions	Benzaldehyde	B4a	*510*
DDT	Insecticides, milk, fatty materials	Nitric acid, sodium methoxide	B6a	*93, 431, 432*
	Technical DDT	Acetic and sulfuric acids	B1a	*78*
	Insecticides	Aluminum chloride	B6a	*17*
Deguelin	Cube and derris roots	Sulfuric acid, nitrous acid	B7a	*247*
1-Chloro-2,4-dinitrobenzene	2,4-Dinitroanisole	Pyridine, alkali	B4a	*430*
Diethylstilbestrol	Tablets	Molybdiphosphoric-tungstiphosphoric acids	B2g	*508*

TABLE 1·8 METHODS FOR ORGANIC CONSTITUENTS (*Continued*)

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
Dihydrorotenone	Rotenone materials	Nitrite, sulfuric acid	B6a	*184*
2,4-Dinitroanisole	Insecticides	Potassium cyanide	B1a	*429*
Egg yolk (cholesterol)	Egg white	Acetic anhydride, sulfuric acid	B4a	*102*
Ergosterol	Solutions	Sulfuric acid	B4a	*381*
Flavanones	Citrus fruits	Diethylene glycol, alkali	B4a	*113*
Formaldehyde	Other aldehydes, formals	Schiff's reagent	B4a	*38, 226*
	Blood	Molybdiphosphoric-tungstiphosphoric acids	B2g	*193*
	Water	Chromotropic acid	B4a	*55*
Fuel components	Liquid fuels	Phenazine dyes	B5a	*245*
Furfural	Plant materials	Aniline acetate	B4a	*128, 272, 487*
Gallic acid	Fats, oils	Ferrous tartrate	B2a	*303*
Glycolic acid	Fats	Dihydroxynaphthalene	B4a	*70*
Gossypol	Cottonseed oil, meal	Aniline	B4a	*295, 470*
	Cottonseed, meal	Antimony trichloride	B4a	*40*
Hemicellulose	Wood-pulp liquors	Dichromate	B1a	*27*
Hemoglobin	Meat scraps, tankage	....	A	*393*
Hydrocarbons, aromatic	Water	Formaldehyde, sulfuric acid	B4a	*341*
	Air, water	Nitric acid, alkali, butanone	B6d	*16*
Indole	Butter	Dimethylaminobenzaldehyde	B4a	*81*
Lactic acid	Blood	Veratrole	B4a	*137*
Lipoid oxidase	Plant materials	Ferrous thiocyanate	B1a	*496*
Methanol	Solution	Permanganate, chromatropic acid	B1d	*146*
2-Methyl-1,4-naphthoquinone	Drugs	2,4-Dinitrophenylhydrazine, ammonia	B4a	*323*
Mononitroparaffins	Air	Ferric chloride	B2a	*442*
Nicotine, Nornicotine	Air, tobacco	Cyanogen bromide	B6a	*305, 283*
Nicotine	Insecticides	Tungstisilicic acid	T	*183*
Nitrate esters	Biological materials	*m*-Xylenol, sulfuric acid	B6a	*556*
Oils	Water emulsions	....	F	*32*

TABLE 1·8 METHODS FOR ORGANIC CONSTITUENTS (*Continued*)

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
Oxalic acid	Fats	Dihydroxynaphthalene	B4a	*70*
Oxidation	Quenching oils	. . . . .	A	*88*
Penicillins	Fermentation liquors, salts	Hydroxylamine, ferric chloride	B4b	*161*
		Assay organisms	T	*217*
	Calcium penicillin solutions	Sodium nitrate, *staphylococcus aureus*	B1h	*189*
Pentachlorophenol	Fabrics, fungicides	4-Aminoantipyrine	B4a	*190*
	Biological materials	Nitric acid	B6a	*116*
Peroxides	Unsaturated oils, rubbers	Ferrous thiocyanate	B1b	*401, 513, 571*
	Rubber, polymers	Ferrous sulfate, 1,10-phenanthroline	B1b	*277*
Phenol	Hydrocarbons	Nitric acid	B6a	*530*
	Petroleum products	Nitrous acid	B6a	*294*
	Water	2,6-Dibromoquinonechloroimide	B4a	*36*
Phenothiazine	Sprays, residues	Palladous chloride	B2a	*366*
Polyethylene glycols	Biological materials	Molybdiphosphoric acid	B2g	*450*
Purity	Alcohol	Permanganate	B1c	*392*
Proteins	Biological materials	Molybdate	B2g	*386*
Quercitin-like substances	Plant materials	Boric and citric acids	B2a	*542*
Quinone oximes	Reaction mixtures		A	*204*
Resins	Hops	Uranyl nitrate	B2a	*61*
Rotenone	Insecticides	Thymol, hydrogen peroxide	B4f	*405*
	Cube and derris roots	Sulfuric and nitrous acids	B7a	*247*
Salicin	Carbohydrate materials	Sodium hydroxide	B7a	*241*
Sesamin	Sesame oil	Perchloric acid, hydrogen peroxide	B1a	*243*
Starch	Paper	Iodine	B2a	*467*
	Plant materials	Iodate-iodide	B2a	*356, 388*
Sterols	Leaf meals	Acetic anhydride, sulfuric acid	B4a	*517*
Sucrose	Solutions	Anthrone, sulfuric acid	B4a	*343*
Sugars, reducing	Plant materials	Potassium ferricyanide	B1c	*162, 337*
Sulfonamides	Blood	Nitrous acid, N-(1-naphthyl)-ethylenediamine	B3a	*284*

TABLE 1·8 METHODS FOR ORGANIC CONSTITUENTS (*Continued*)

Desired Constituent	*Sample*	*Reagent*	*Type*	*References*
Tannin substances	Boiler water	Molybdiphosphoric-tungstiphosphoric acids	B2g	*35*
Tetraethyl lead	Air	Silver nitrate on silica gel	B1a	*67*
Trinitrobenzene	Dinitrobenzene	Sodium hydroxide	B5a	*345*
Trinitrotoluene	Water	Sodium sulfite, alkali	B5a	*411*
Urea	Upholstery, bedding	Urease, Nessler's reagent	B2a	*390*
Vanillin	Extracts	*o*-Iodoxy ammonium benzoate	B4a	*111*
Xanthone	Spray residues	Sodium amalgam, hydrochloric acid	B6a	*75*
		Vitamins and Related Materials		
Vitamin A	Biological materials	Direct measurement in ultraviolet and visible	A	*228, 363, 365, 535, 574*
	Biological materials	Antimony trichloride	B4b	*228, 363, 365, 47, 53, 103, 170, 172, 179, 364, 407, 504*
	Fish-liver oils	Glycerol dichlorohydrin	B4a	*475, 476*
Carotenes	Plant materials	... .	A	*42, 65, 130, 166, 296, 205, 360, 382, 457, 572, 573*
Vitamin B				
Thiamin	Biological materials	.	F	*91, 118*
Riboflavin	Biological materials		F	*118, 410, 522*
	Dried milk		A	*493*
Niacin, niacinamide	Biological materials	Cyanogen bromide, aniline	B4a	*280, 321, 322, 514*
	Biological materials	Cyanogen bromide, *p*-Aminoacetophenone	B4a	*9*
Vitamin C (ascorbic acid)	Biological materials	2,6-Dichlorophenolindophenol	B1c	*72, 224, 290, 338, 354*
	Foods, fruit juices	Iron, bipyridine	B1a	*264*
Vitamin D	Biological materials	Glycerol dichlorohydrin	B4a	*477*
	Biological materials	Antimony trichloride	B4a	*90, 120, 281, 326, 451*
Vitamin E (tocopherols)	Vegetable oils, plants	Nitric acid	B1a	*157, 516*
	Vegetable oils, plants	Ferric chloride, bipyridine	B1b	*485, 516*
	Mixtures	Ferric chloride, bipyridine, diazotized *o*-dianisidine	B1b, B3a	*523*
Vitamin K (synthetic)	Pharmaceuticals	Sodium pentacyanoammine ferroate	B2a	*324*

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2. PHYSICS

General Principles of Absorptimetric Measurements

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A·INTRODUCTION

It is pointed out in Section 2·1 that absorptimetry is one of a number of different kinds of methods used in quantitative chemical analysis to determine, by measurement of a physical property, what a constituent is, its amount, or simply the magnitude of a property as such. Whatever the objective may be, the property concerned in this case is the absorptive capacity of the sample system for radiant energy of some specified wavelength(s). If the measurement is confined to the visible region of the spectrum, it is the light absorptive capacity which is determined. With an aqueous solution of copper (II) sulfate as the sample, the final objective might be the determination of the amount of the copper in the solution, the specification of the color of the solution, or both.

2·1 The place of chemistry, physics, and spectrometry. To judge from various oral and written expressions of spectroscopists, there is confusion over the role of analytical spectrometry in chemical analysis and over the part played by chemistry and by physics in any analytical method. Consequently, a brief statement seems appropriate in order to orient the reader on this point.

Analytical chemistry deals with the determination of what constituents are in a given sample and/or the amounts of the desired constituents. Depending upon the objective, we have, respectively, qualitative and quantitative analysis. Closely allied to actual analysis in modern testing and analytical laboratories is the measurement of physical properties in order to characterize materials, to predict performance, or to set specifications. Thus, the steel laboratory may determine the percentage of manganese in a steel and also the physical properties of the steel, such as hardness and tensile strength.

Our immediate concern is how absorption spectrometry fits into this picture. In order to decide the question one needs some perspective of the current status of the general subject. For simplicity, the discussion is limited to quantitative methods. An elaboration of the outline has been presented elsewhere (*30*).

Obviously, regardless of the method of testing or analysis, there must be a sample. Since one wants the amount of one or more desired constituents or the magnitude of some physical property, there must follow an appropriate measurement.

It may be stated, probably without question, that the industrial objective is to make this measurement on the sample as such. This is the simplest thing to do and, consequently, the most rapid and economical. Examples are the titrimetric determination of the acidity of vinegar, the densimetric determination of the ethanol in a radiator solution, and the absorptimetric determination of the color of a plastic material.

Samples as such serve for the determination of the large majority of physical properties and also for the chemical composition of many substances; but the determination of the amounts of constituents in vast numbers of samples, by a variety of methods, requires more or less treatment before measurement of the desired constituent. Consideration has been given in Chapter 1 to some of the kinds of problems often encountered in the preparation of a sample for colorimetric measurement of a desired constituent. The presentation includes a general outline of important kinds of chemical transformations which may be necessary in order that measurement of the system derived from the sample may yield the information desired. All such treatment is very largely *chemistry.* Its prime importance is not always appreciated by those who have not had adequate training and experience in general quantitative analysis. Such individuals may be unmindful of the fact that mere refinement of measurement and elaboration of gadgetry in the instruments will not in themselves take the place of, or compensate for, inadequate selection of the sample or inappropriate preparation and handling of the portion of material submitted for analysis, or of the standards used to calibrate the measuring instrument. The analysis of granite rocks is a superb example of the significance of chemical transformations in making possible the required measurements. The standard established for this work by H. S. Washington, a total of 100.00 ± 0.25 per cent for some twenty constituents, should not be forgotten by modern instrumentalists.

Until analytical methods progress beyond their present state, the application of more or less chemistry will be necessary in great numbers of cases. The continued importance of this chemical step prompted not only the inclusion of Chapter 1 but also the location of this discussion at the beginning of the book.

When the sample is ready for measurement, whether as such or in the form of a system appropriately prepared by methods such as those outlined in Chapter 1, one comes to the *physics* stage of the analytical procedure. By definition, measurement is physics, since essentially the operation consists in determining the number of times the standard of measurement goes into the unknown. It may be mentioned, of course, that inadequate knowledge of the measuring instruments or improper attention to details in handling them on the part of a chemist is just as productive of unreliable results as is the lack of understanding of chemistry on the part of a physicist.

At the point of measurement there arises the question of which property to measure and how to do it. In order to locate absorptimetry and colorimetry, it will suffice here briefly to consider some properties, the measurement of which will yield analytical data. Specific instrumentation and methods of operation belong in several subsequent chapters.

As in any other kind of measurement, the measurable properties are physical. It may help to classify them as specific and systemic. With specific property methods one measures a property of the *desired constituent itself*, or of some other substance in terms of which the amount of the desired constituent may be calculated. For example, one precipitates calcium as the oxalate, dissolves the precipitate, and titrates the oxalate ions; then each oxalate ion is assumed to be equivalent to a calcium ion. The following list includes important examples, together with a name appropriate to indicate the property measured: *

Mass: gravimetric (gravimetry).
Volume: volumetric (volumimetry).
Pressure: manometric (manometry).
Chemical reactivity
  Combination capacity: titrimetric (titrimetry).
  Heat of reaction capacity: calorimetric (calorimetry).
  Radiation capacity
    Natural: radiometric (radiometry).
    Excited: emission spectrometric (emissimetry).

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* Incidentally, this functional nomenclature renders unnecessary the usage of terms such as those designating physical state (*wet*, *dry*, *solid*, *liquid*, *gas*); noncoordinate terms, such as *spectrochemical* (we do not have comparable terms, such as *gravichemical*); and nondifferentiating words, such as *instrumental* (all measurements involve instruments, and hence are instrumental; the analytical balance is just as much an instrument as is a photometer).

To be strictly consistent, all such words should end in *-imetry* or *-imetric*. Because of either long usage (*manometric*, *potentiometric*) or possible objection to having a word with *ii* (as *radiimetric*), a few spellings here are inconsistent. Possibly one might simply omit the *o* in words such as *radiometric* and *potentiometric*.

In contrast to these methods, with systemic methods one measures a property of the system *containing the desired constituent,* or something chemically equivalent to the desired constituent. Again, the following list includes important examples, along with names:

Density: densimetric (densimetry).
Optical properties
  Refractivity: refractimetric (refractimetry).
  Turbidity: turbidimetric (turbidimetry).
  Absorptivity: absorptimetric (absorptimetry).
  Color: colorimetric (colorimetry).
Electrical properties
  Conductivity: conductimetric (conductimetry).
  Potential: potentiometric (potentiometry).
  Current: currentimetric (currentimetry).
Thermal properties: thermimetric (thermimetry).
Colligative properties: colligimetric (colligimetry).

For decades chemists have used the term *colorimetry* to refer to methods, such as those discussed in Chapter 3, in which the measurement is accomplished by comparing the color of the system of unknown concentration with that of a system of known concentration (or definite color value). The absorbing capacity of the unknown is thus compared with that of the standard by means of instruments called *comparimeters* or simply *comparators.* It is for such individuals that provision is made in the outline above for *colorimetry.* Physicists, however, think that the term *colorimetry* should be restricted to the kind of determination described in Chapter 9. Such measurements may be considered as a determination of color as color, or of color specification, in terms of equivalent stimuli.

As already mentioned, absorptimetric methods are based upon the measurement of a systemic property, in this case the absorptive capacity of the sample for radiant energy. When the absorption is selective in the visible region of the spectrum, the object appears colored to the normal human eye. Chapters 4 to 8 deal with the use of ultraviolet, visible, and infrared absorptimetric methods for the determination of the nature of constituents and/or their amounts in a given sample. Finally, in Chapter 9, comes the use of absorptimetry (reflectimetry), both relative and absolute, for the determination of the color of objects.

It is evident that absorptimetry is merely one kind of analytical method. The various kinds of absorptimetric methods considered currently most important for making measurements in the spectral region of 0.2 to 25 microns are summarized in this book.

B·TERMS AND SYMBOLS

A considerable technical vocabulary has grown up during the past century or more in connection with absorptimetric and colorimetric work. Now probably no other division of analytical chemistry is more confusing in its definitions, usages, and conventions.

2·2 General principles. Certainly to some extent this situation may be attributed to the varied interests and backgrounds of those who have produced the technical literature. Thus, the Inter-Society Color Council is composed of representatives from a dozen or more different technical societies, such as physicists, psychologists, illuminating engineers, colorists, and artists.* Personal indulgence in too much *laissez faire* individualism and lack of any agreement among countries have played their part. Then, too, there has been no standardizing agency or committee, such as the international committee on atomic weights.†

Rather than try to gather together at this point all definitions and symbols, it has seemed preferable to have each contributor bring in certain terms at the point of discussion, with cross references where necessary. Since a number of words and symbols are used in more than one chapter, consistency in use has been sought.

Since the measurements are physical, and since physicists have recommended certain usage in these matters, especially in the report of the colorimetry committee of the Optical Society of America (*19*) and in *Letter Circular* LC-857 (*33*) of the National Bureau of Standards, these recommendations have been taken here as the general guide. The reader is referred, then, to Chapter 5, Division B, for definitions of terms such as *radiant energy*, *light*, and *color*. Still other terms, especially those concerning color, may be found in Chapter 9, Division F. The symbols used are those given in Chapter 5, except as noted in Chapter 6, where English usage is followed.

The confusion likely to arise when one attempts to use the widely scattered absorptimetric data results from their being recorded in so many different ways, often with different symbols for the same thing. Some of this usage is summarized in Table 2·1. The information was taken from well-known monographs and treatises for comparison with the usage recommended in *Letter Circular* LC-857, the basis adopted for this book.

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* Curiously, the American Chemical Society has never cooperated, although hundreds of its members are engaged in work involving such items.

† It is interesting to contemplate the chaos which might now prevail had this committee not been established to recommend symbols and atomic weights and, in view of its eminently satisfactory work, to consider what might now be operative in absorptimetry and colorimetry with a committee of similar standing. Mention is made, of course, in Chapter 9 of the recommendations of the International Committee on Illumination (I.C.I.).

TABLE 2·1 ABSORPTIMETRIC TERMS AND SYMBOLS

Publication	Transmittancy	Concentration (c)	Thickness, cm	Absorption Coefficient	Extinction Coefficient	Molar Extinction Coefficient	Extinction
Brode (*9*)	I/I_0	g /l.	d		$k = E/cd$	$\epsilon = k$ mol. wt.	$E = \log I_0/I$ $= kcd$
Drabkin (*10*)	I'/I	mol./l.	d		$\epsilon_c{}^d$	E	$\epsilon = -\log T$
Gibb (*13*)	I/I_0	g /l.	l	μ	k		$d = \log I_0/I$ E = density
Henri (*17*)	J/J_0	g mol./l.	l	$\mu(k)$	K	$\epsilon = K/c$	$E = \log I_0/I$
ICT * (*45*)		g. mol /l.	l	μ	$K = d/l$	$\epsilon = K/c$	d = density
Landolt-Bornstein (*37*)	J/J_0 T	g. mol./l.	d	k'	$\alpha = k$	$\epsilon = k/c$	$\log J_0/J$
Ley (*26*)	J/J_0	mol./l.	d	k' $1/d \ln J_0/J$	$k = E/d$	$\epsilon = k/c$	$E = D$ (optical density)
N.B.S. (*33*)	I/I_0 T_s	mol /l.	b		$a_s = A_s/bc$ †	a_M ‡	A_s§ $= \log I_0/I$ $= -\log T_s$
Weigert (*46*)	J/J_0	mol./l.	d	a	$E = \epsilon$	$K = E/c$	
West (*48*)	I/I_0	mol./l.	d	k	K	$\epsilon = D/cd$	$D = \log I_0/I$

* Also Twyman and Allsopp (*42*).
† Absorbancy index.
‡ Molar absorbancy index.
§ Absorbancy.

C·PRINCIPLES OF MEASUREMENT

In general, radiant energy incident upon a nonopaque (transparent) body will be partly reflected, partly absorbed, and partly transmitted. In the case of opaque bodies none is transmitted. The respective *processes* are reflection, absorption, and transmission, the *magnitudes* of which are measured in terms of reflectance, absorptance (or absorbance), and transmittance. To refer in a general way to the capacity of the body to reflect, absorb, or transmit radiant energy, the writer likes to use the respective terms *reflectivity*, *absorptivity*, and *transmissivity*, which are analogous to *conductivity* in electrometric work. As mentioned under definitions, specific meanings have been attached to these words by some (see LC-857 and Ref. *19*).

2·3 Instrumental essentials. To make such a measurement, most or all of the following items are concerned: (1) a source of radiant energy, which, in the visible region of the spectrum, is a light; (2) a container for liquid or gas samples; (3) a receptor for the radiant energy which is to be measured, after reflection or transmission; and (4) accessory means to facilitate the measurement. Suitable combinations of these items in measuring assemblies constitute the instruments used. Application of the methods is based, of course, upon knowledge of the laws of absorption and an understanding of conventions used in handling the data involved. Since the details for most of these items differ widely for the different kinds of methods discussed in subsequent chapters, such matters are presented there. Consideration is limited here to those aspects which seem of general introductory interest.

2·4 Sources of radiant energy. In order to determine the absorptive capacity of a given system for radiant energy, or the color of an object, a suitable source of such energy is required. Since many sources, having a wide range of characteristics, are available, and since the results of a measurement depend upon these characteristics, some understanding of sources is necessary. Figure 2·1 shows the approximate spectral range covered for various kinds of rays and waves, including those discussed in this book. Rather generally, reference is made to the ultraviolet, visible, and infrared regions of the spectrum, the over-all range considered here being approximately 0.2 to 25 microns. Neither these limits nor those for the visible region are sharply defined. The latter, of course, are a function of the observer; but for a person with normal vision the visible region extends approximately from 400 to 700 mμ.

Sources are important from the viewpoint of a number of factors, such as the range, the relative spectral distribution in this range, the means of production, the stability, and the operating temperature.

As a matter of ultimate desire, probably one would like to have, in one direction, a strictly monochromatic source, with the emitted line locatable at the single wavelength desired, and, in the other direction, a continuous source usable at any and all wavelength regions. Practically, neither is available. The various line sources, such as the mercury arc and the helium discharge tube, radiate at a number of wavelengths, giving lines of unequal intensity (see Chapter 5). Suitable filtering or monochromator settings may let pass the energy of a single line. Thus, calibration data for glasses from the National Bureau of Standards may give transmittances at wavelengths such as 404.7, 435.8, 546.1, and 690.7 mμ. If so, they are readings taken with the König-Martens instrument, using the mercury lines of the respective wavelengths.

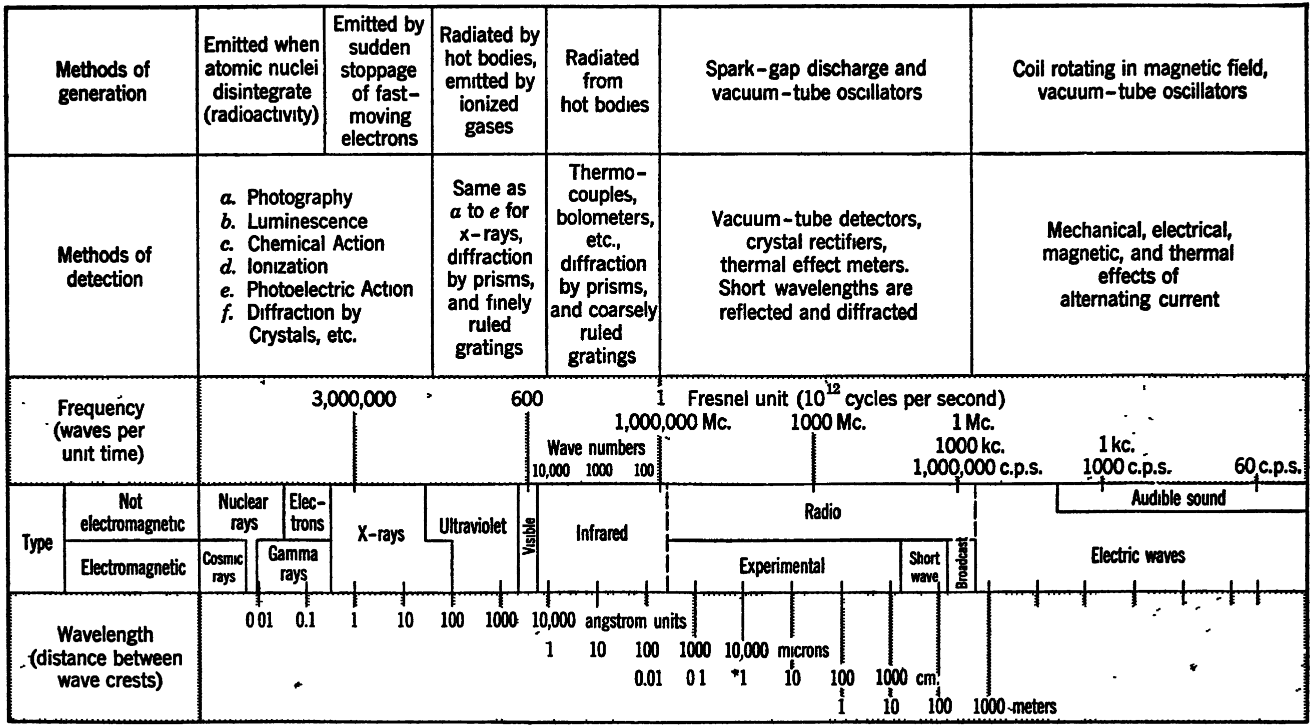

FIG. 2·1. The electromagnetic frequency spectrum, with upper boxes showing how various portions of the spectrum can be generated and detected for use in the analytical field. [Reproduced from *Electronics*. **17,** 116, 308 (1944), by permission of the authors, F. P. Hochgesang and C. H. Schlesman, and of the McGraw-Hill Publishing Company.]

No single continuous source is available for the region 0.2 to 25 microns. Some well-known sources covering part of the range include the following: ultraviolet—the hydrogen arc and, formerly, the underwater spark; visible—sunlight and the incandescent tungsten bulb; and infrared—the Nernst glower and the "Globar" lamp. Even these sources vary in their effectiveness at different wavelengths and under different operating conditions. Thus, light from a tungsten incandescent bulb at a temperature of 2850°K. is more intense at 700 than at 400 mμ. Then at each different temperature the situation changes. Details of these and other sources are given in the appropriate chapters.

Stability of the output is very desirable. In fact, it may be indispensable, as in instruments in which the measurement is the galvanometer response to the incidence of the light beam upon a photocell. There is involved, therefore, the instrumental problem of stabilization, automatic regulation, and auxiliary compensation for fluctuations.

Modulation of the radiant energy from the source is a requirement in some instruments. That is, the beam is interrupted regularly and rapidly by means of a chopper, a flicker system, or a pulse source. For example, the two beams in the General Electric spectrophotometer (see Chapter 5) are alternated 1800 times a minute by means of the rotating rochon prism in the shaft of the synchronous motor.

Second only in importance to the source itself are the means usually employed to limit the radiant energy incident upon the sample to some particular part of the spectrum. Such means vary from simple filters passing wide ranges to the finest slit assembly in a monochromator. Thus, from a line source a single spectrum line may be isolated in certain instances; but, from a continuous source, the best possible is a relatively narrow band of the spectrum, generally not better than 1 mμ in the visible region. It is to this property that one refers in using the expression *spectral-band width.* This is not synonomous with *slit width* for the monochromator.

2·5 Containers. Except in special cases, such as finely divided powders, containers are not a problem for solids. Liquids and gases, however, must be held in something suitable during measurement. In general, this means a container or absorption cell of some kind which will transmit the radiant energy over the range of measurements desired. Common examples are fused quartz for the ultraviolet, optical glass for the visible, and various alkali salts for the infrared. It will be noted, of course, that the materials used for the optical parts of the measuring instrument correspond to the kind used for the cells.

The form of absorption cell depends chiefly upon the instrument used. It varies from a simple test tube, used for visual observation or in simple filter photometers, to multiple reflecting cells, used for infrared work with gases. When temperature is an important factor, jacketed cells may be necessary. Several concerns have a wide variety of simple and precision cells available (see Ref. *2*).

The quality of material should be adequate for the accuracy of measurement desired. For the best spectrophotometric work the materials should meet specifications of high-quality optical cells, such as glass uniformly clear and polished to plane, parallel faces. For liquids the most convenient cells have fused-on faces or ends. If the beam path is short, as with highly absorbing materials, the end pieces may be held in place mechanically. The best cells represent fine workmanship.

Meticulous cleanliness and handling are no less important than cell quality in high-precision work.

2·6 Receptors. Just as the container must pass radiant energy through the wavelength region where measurements are desired, the receptor must be capable of detecting and receiving the unabsorbed part of the energy. Likewise, just as there is no usable universal source, there is no single satisfactory receptor for the whole region. The ones available may be subdivided into nonselective and selective types.

The nonselective type is represented by thermocouples, thermopiles, and bolometers. Although research continues for better sources of this kind, thermocouples and bolometers seem to be preferred for the infrared region.

Various selective sources are the preferred means for the visible and the ultraviolet regions. The human eye, of course, is *the* means for visual observation, as described in Chapter 3. The photographic film or plate covers the ultraviolet and, if sensitized, much of the visible region. The development of photocells, especially the photoemission and photovoltaic types with different ranges and sensitivities, has revolutionized instrumentation in this field.

Each kind of detector, in addition to the limitation on spectral range covered, possesses other characteristics which must be kept in mind when using an instrument so equipped. Thus, visual instruments, whether comparimeters, filter photometers, or spectrophotometers, involve the unavoidable low sensitivity of the human eye under 450 and above 675 mμ. Certain photocells may have considerable lag and fatigue effects. Various details are mentioned subsequently in the appropriate chapters.

In the course of instrumentation involving different kinds of receptors, an array of auxiliary devices has come into use, especially with photocells, thermocouples, and bolometers. These include, among other items, amplifiers, means of signal conversion for purposes of recording, indicators, and recorders.

2·7 Instruments. Absorptimeters consist ultimately, then, of some combination of radiant-energy source, container, and receptor, combined frequently with some mechanical or other devices for facilitating acquisition of the desired data. Examples of such devices are filters, monochromators, photometers, and recorders. Only a comprehensive book could give details on all the known possibilities of instruments alone. Thus, considered from the standpoint of the spectrometry, geometry, and photometry involved, there are 144 combinations for spectrophotometers (*18*). This number does not include other types of instruments. One of the simplest in the range covered would be visual matching of two test tubes illuminated with sky light. Perhaps the most complicated thus far would be Zscheile's nonrecording spectrometer (*52*) and the Baird recording infrared spectrometer (*5*).

D·THE PROCESS OF ABSORPTION

Monographs in physical chemistry deal in detail with the broad aspects of the process of absorption of radiant energy by substances. The interest here is entirely analytical applications.

2·8 Nature of the process. Because of the expressed limitation of this book, we shall consider the nature of the process of absorption only in so far as there is interest in an interpretation of the origin of spectra in the ultraviolet, visible, and infrared regions of the spectrum. The statement following is quoted from an elementary textbook of physical chemistry.*

> These different methods of absorbing radiation in the different parts of the spectrum and the energies involved are summarized in Fig. 2·2.
>
> In the upper half of the figure are given the general types of spectra together with the wavelength regions and the energy of the radiation in calories per mole. In the lower part is given a crude representation of what happens when the radiation is absorbed. The black dots represent electrons, and the larger circles represent atoms in the molecule. The same general considerations apply to polyatomic as well as to diatomic molecules. All the intramolecular changes involve definite energy increments which are governed by the quantum theory.
>
> Absorption in the far infrared causes the molecule to rotate as indicated by the curved arrow at the right. The energy involved in these operations is of the order of a thousand calories per mole.
>
> This is the only operation which can be carried out with energies of this magnitude, and so there are definite lines corresponding to quantum numbers. Ab-

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* Reprinted by permission from *Outlines of Physical Chemistry*, by Farrington Daniels, copyrighted 1948, John Wiley & Sons, Inc.

sorption in the near infrared displaces atoms from their normal positions and causes them to oscillate back and forth or to move sidewise with a swinging motion within the molecule. These operations involve energies between about 1000 and 30,000 to 40,000 calories. The rotations, involving small energies, are superimposed on the atomic displacements, giving rise to absorption bands. The intramolecular changes can be combined so as to give harmonics absorbing approximately two or three or more times as much energy as the energy of the fundamental absorption band and causing absorption bands at roughly one-half and one-third the wavelength. The probability of absorbing energy corresponding to two or three or more quanta is less than the probability of absorbing one, and so the fundamental band (the one-quantum operation) is the most prominent, and the absorption becomes progressively less intense at the higher harmonics. This decrease in intensity at the shorter wavelengths is represented diagrammatically by reducing the height of the bands.

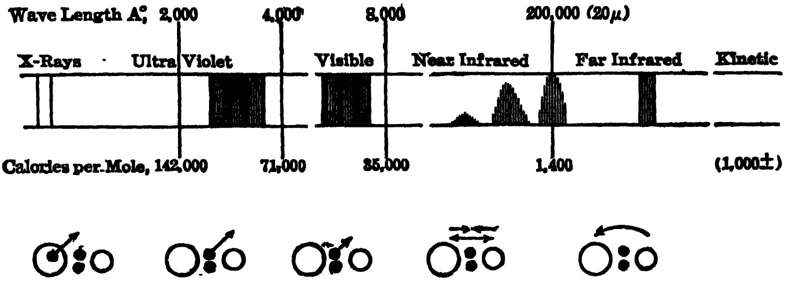

Fig. 2·2. Schematic representation of different types of spectra showing wavelength ranges and energy ranges in which they occur, and the corresponding electronic, vibrational, and rotational motions. (Reproduced by permission from *Outlines of Physical Chemistry*, by F. Daniels, John Wiley & Sons, 1948.)

In the visible and ultraviolet region of the spectrum the absorption consists in displacing an outer electron in the molecule. The only difference between the ultraviolet and the visible spectra is that greater energies and larger displacements are involved in the ultraviolet absorption—35,000 to 71,000 calories per mole being required for absorption in the visible region and 71,000 to several hundred thousand for absorption in the ultraviolet.

Sometimes the energy of the ultraviolet is sufficient not only to displace the electron within the molecule but also to drive it entirely out of the molecule, thus producing ionization. In the visible and ultraviolet regions of the spectrum series of bands may be obtained by combining the energies of the three quantum-restricted operations—electron displacement, atomic displacement, and molecular rotation. . . .

The various absorption spectra, produced by the displacements indicated in Fig. 2·2, are usually the same as the emission spectra produced when the electrons or atoms return to their normal positions, or when the molecules are caused to rotate by collisions or other means.

At the extreme right of Fig. 2·2 is given for comparison the order of magnitude of the average kinetic energy of molecular translation $\frac{3}{2}(RT)$, a few hundred calories.

2·9 The laws of absorption. The laws of absorption of radiant energy with which the analyst is concerned for solutions involve the relationship between the magnitude of absorption and the amount of the absorber. Experimental study of this absorption (originally of light) by homogeneous, transparent (nonopaque) solids, liquids, and gases led to the formulation of two laws representing relationships of much importance in absorptimetry. Since the application of these laws is not limited to any particular kind of method or instrument, the basis and significance of the generalizations should be considered before taking up subsequent chapters.

2·10 Bouguer's law. The first of these laws, formulated by Bouguer (7) but often attributed to Lambert, expresses the relationship between absorptive capacity and thickness of the absorbing medium. According to this law, each layer of equal thickness absorbs an equal fraction of the radiant energy which traverses it. Mathematical statements relating the quantities involved are found in two forms—logarithmic and exponential. They are based on the respective statements that the absorptive capacity varies directly as the logarithm of the thickness, and that the intensity of the transmitted energy decreases exponentially as the thickness of the absorbing medium increases arithmetically.

In the statements of the relationships, use will be made of the symbols and definitions given in Chapter 5 (see Fig. 5·1). I_0 represents the energy (or intensity of that energy, as some prefer) entering the sample in the absorption cell; I, the energy leaving the sample and incident upon the second surface of the absorption cell; b, the thickness of the layer of the absorbing solution; c, the concentration of solute in the solution; and T_i the (internal) transmittance, or I/I_0. It may be noted that the absorptive capacity of the absorption cell and reflectances at its surfaces are disregarded, since in practice their effects are compensated by standardizing under working conditions.

The logarithmic statement of the law will be considered first. The absorption of energy (the decrease in intensity per unit thickness) is proportional to the intensity of the energy. Expressed mathematically,*

$$-\frac{dI}{db} = kI \tag{2·1}$$

---

* This derivation and the accompanying examples are adapted by permission from *Mathematical Preparation for Physical Chemistry*, by Farrington Daniels, copyrighted 1928, McGraw-Hill Book Co.

In this equation, I is the energy after passing through a layer of absorbing medium b units thick. Then,

$$-\frac{dI}{I} = k\,db \tag{2·2}$$

Integrating between the limits I and I_0 and b and 0, the following equation is obtained,

$$\int_{I_0}^{I} -\frac{dI}{I} = \int_0^b k\,db$$

$$-[\log_e I]_{I_0}^{I} = k[b]_0^b$$

$$k = \frac{2.303}{b}[\log_{10} I_0 - \log_{10} I] = \frac{2.303}{b}\log_{10}\frac{I_0}{I} \tag{2·3}$$

I is the energy after passing through b cm. (or any other unit) of absorbing material, and I_0 is the energy when $b = 0$.

Example. If the energy is reduced by 10 per cent in passing through 1.00 cm. of a solution, giving an intensity of 1.00 − 0.10, or 0.90, what will be the energy after passing through 10.0 cm.?

$$k = \frac{2.303}{1}\log_{10}\frac{1.00}{0.90} = 2.303 \log_{10} 1.111 = 2.303 \times 0.0457 = 0.10525$$

Having established the value of k from two experimental observations (intensities at two different depths, zero and b), the intensity, I, at any value of b may be calculated. Thus, when $b = 10$,

$$0.10525 = \frac{2.303}{10}\log_{10}\frac{1.00}{I} = -0.2303 \log_{10} I$$

$$\log_{10} I = \frac{-0.10525}{0.2303} = -0.457$$

$$I = \text{antilog}\ \bar{1}.543 = 0.349$$

The intensity is thus reduced to 34.9 per cent of its original value after passing through 10 cm. of material.

One may want to know the thickness required to reduce the intensity a given amount. Thus, through what thickness would a beam have to pass before the intensity is reduced by 90 per cent (reduced to an intensity of 10 per cent)?

$$0.10525 = \frac{2.303}{b}\log_{10}\frac{1.00}{0.1}$$

$$b = \frac{2.303}{0.10525} \times 1 = 21.9 \text{ cm.}$$

We may now consider the exponential expression of the relationship involved (*15*). If a layer of unit thickness transmits a fraction t of the energy incident upon it, a thickness b will transmit the fraction t^b. Then,

$$I = I_0 t^b \tag{2·4}$$

This law may be expressed in the form,

$$T_i = e^{-k'b}$$

in which T_i is the transmittance (I/I_0), e is the base of the natural system of logarithms, and k', which varies with the wavelength of the incident radiant energy, is the absorption coefficient. In the new nomenclature, this constant presumably would be a_i', although no provision was made for ln-type expressions, and no names were assigned.

Using Briggsian logarithms, the expression becomes,

$$T_i = 10^{-kb} \tag{2·5}$$

In this form the constant k has been known as the extinction coefficient. Instead, the LC-857 designation (*33*) is a_i, known as the absorbance index.

As an application of this law, a special slide rule * enables one to calculate from a known transmittance and thickness to an unknown transmittance at a different thickness. For example, values may be desired for 30-cm. Nessler tubes, but the apparatus available provides for the determination of data for thicknesses only up to 5 cm. However, as pointed out by Hardy (*15*), this calculation cannot be made for transmittance (e.g., glass) without correction for the losses at the two air-glass interfaces, each of which causes a loss of approximately 4 per cent.

Example. Suppose that the transmittances at various wavelengths are given for a thickness of 1.47 cm. and that it is desired to find the corresponding transmittances for a thickness of 1.27 cm.

Use is made of the transmittance scales b_1 and b_2 on the back face of the rule (actually labeled t_1 and t_2 on the author's rule). In the expression

$$(T_i)_1^{1/b_1} = (T_i)_2^{1/b_2} \tag{2·6}$$

$(T_i)_1$ is the transmittance at a given wavelength of the measured sample, and b_1 its thickness; b_2 is the thickness of the sample whose transmittance, $(T_i)_2$, is desired.

Set the slide of the rule so that 1.27 on the B scale is in line with 1.47 on the A scale. Then on the b_1 scale or the b_2 scale on the rule set the indicator on the known transmittance for thickness 1.47, and read off the computed transmittance for thickness 1.27 on the corresponding scale on the slide. Thus, for thickness 1.47 and transmittances 96.0, 33.1, 23.1, 11.9, 4.9, the transmittances for thickness 1.27 become, respectively, 96.5, 38.4, 28.2, 15.8, 7.4.

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* Keuffel and Esser Co.; appropriate scales on other slide rules will serve the same purpose.

2·11 Beer's law. The second law, formulated by Beer (*6*), expresses the relationship between absorptive capacity and the concentration of the solute in a solution. According to this generalization, the absorptive capacity is directly proportional to the concentration of the solute (number of absorbing entities of the absorbing substance). Then, if c is the concentration, the transmittance, T_i, for a given thickness is,

$$T_i = t^c \tag{2·7}$$

in which t is the transmittance for a solution of the same thickness having unit concentration.

For solutions conforming to this law, equation (2·6) may be applied, with the substitution of concentrations c_1 and c_2 for thicknesses b_1 and b_2.

2·12 Combined Bouguer-Beer laws. One may combine the laws of Bouguer and Beer for thickness b and concentration c,

$$T_i = t^{bc} \tag{2·8}$$

t being again the transmittance for a system of unit thickness and concentration.

This expression may be written,

$$T_i = e^{-k'bc} = 10^{-kbc} \tag{2·9}$$

Since $T_i = I/I_0$,

$$I = I_0 e^{-k'bc} = I_0 10^{-kbc} \tag{2·10}$$

In the logarithmic form, more familiar to chemists, for unit thickness we have,

$$\log_e \frac{I}{I_0} = -k'c = \log_e T_i \tag{2·11}$$

$$\log_{10} \frac{I}{I_0} = -kc = \log_{10} T_i \tag{2·12}$$

$$\log_{10} \frac{I_0}{I} = kc = \log_{10} \frac{1}{T_i} \tag{2·13}$$

And for thickness b,

$$\log_{10} \frac{I_0}{I} = kbc = \log_{10} \frac{1}{T_i} = A_i \tag{2·14}$$

$$c = \frac{\log_{10} 1/T_i}{kb} \tag{2·15}$$

It may be repeated here that, in the new symbolism of LC-857, the constant k becomes a_i, and the name changes from extinction coefficient to absorbance index.

Equation (2·15) is the basis for calculation of the concentration, having measured the transmittance, having determined the value of k for a solution of known concentration for thickness b, and knowing the value of b.

2·13 Validity of the Bouguer-Beer law. Bouguer's law is an example of the rare generalization which is strictly general in its applicability. It has been stated that there is no known exception to this law for homogeneous systems. In view of this statement, any nonconformity of a system with the combined laws must be attributed to deviation from Beer's law.

A simple experimental test for such conformity is suggested in Chapter 3 by means of the Duboscq comparimeter, since the calculation with such an instrument presupposes conformity. As a more generally useful test today, a set of spectrophotometric curves (see Chapter 5), such as those shown in Division E, will serve to test a given system. First the transmittancies (see Fig. 2·7) or the absorbancies (see Fig. 2·9) for the different concentrations of solution are noted, respectively, at the wavelength of minimum transmittancy or of maximum absorbancy, or as near this point as feasible, if it lies outside the wavelength range of the curves. Then one usually plots as abscissas the concentrations of the solutions, and as ordinates the transmittancies or the absorbancies on a linear scale, or the transmittancy on a logarithmic scale. Straight lines through the individual points for the logarithmic values show conformity, a fact that need ordinarily merely be stated in a paper; if such a curve is to be used for calculations based upon the additive nature of absorbancies, the data should be determined with the instrument used and not taken from a publication reporting the use of another instrument, or even the same kind under slightly different conditions (see Data and Conventions, Division E).

Figure 2·3 shows, for the permanganate curves of Fig. 2·7, (1) the transmittancy-concentration curves taken at the three wavelengths, 525, 500, and 475 mμ, and (2) the corresponding two sets of Beer's law curves. One of these is based on transmittancies and the other on absorbancies. It may be noted that the greatest change in transmittancy or absorbancy per unit change in concentration occurs with the curves for readings taken at minimum transmittancy, 525 mμ (see Division E). An indication of deviation from the law is a change in the absorbancy index with change in concentration. From equation (2·15), $a_i = A_i/bc$, and under constant external conditions (such as temperature and solvent), the value should be dependent only upon the wavelength of radiant energy used.

Nonconformity of a solution to Beer's law indicates the desirability of making comparimetric measurements (see Chapter 3) with a constant-depth standard series method rather than with a variable-depth instrument, such as the Duboscq type. Such deviation may be valuable qualitative evidence of some chemical action affecting the nature of the absorbing entities.

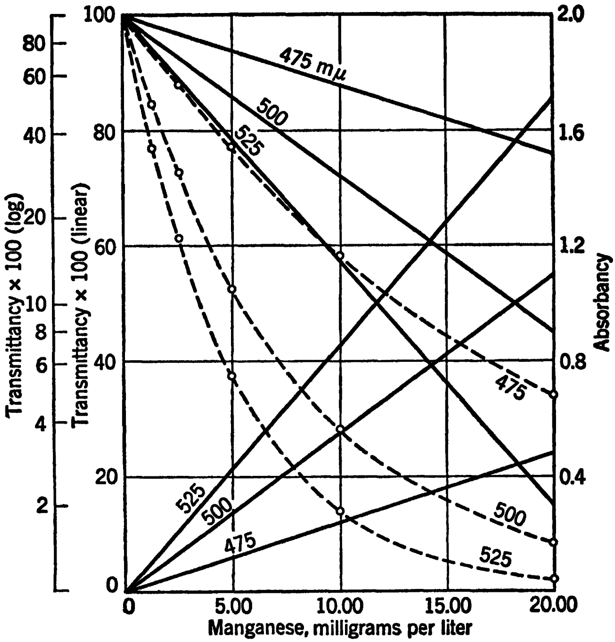

FIG. 2·3. Transmittancy and Beer's law curves for solutions of potassium permanganate at three wavelengths.

Although some individuals seem to have unbounded confidence in the general applicability of Beer's law, as a matter of fact deviations are both frequent and significant (*22*). In summarizing evidence on this point, Körtum (*21*) stated that "the validity of Beer's law for ions must be considered as an exception rather than as a rule. Validity over large concentration ranges is not to be expected since Beer's law is a limit law for low concentrations."

Such disillusionment of one's confidence in a law merits consideration of the causes of divergence. Experimental work indicates two kinds of possibilities—chemical, and physical or instrumental.

2·14 Chemical causes of deviation. A number of careful investigations on the validity of Beer's law have been carried out, starting as early as 1873 (*44*). It is generally concluded that the deviations found are more a result of chemical changes affecting, or occurring in, the absorbing particles than of failure of the law (*43*). Körtum and Seiler have summarized such causes of divergence from the law, and the following discussion is based mainly upon one of their papers (*23*).

Beer's law is only a limiting law for very dilute solutions, and the range in which it is valid is limited even under the most nearly ideal conditions. According to the theory of light dispersion, it is not a_i itself (k in the paper cited), but the quantity $a_i n/(n^2 + 2)^2$ which is a constant, essentially independent of the concentration. As the refractive index, n, generally increases with concentration, a_i must also increase if the expression $a_i n/(n^2 + 2)^2$ is to remain constant. Actually, for concentrations less than 0.01 M the change of refractive index rarely exceeds the accuracy of the measurement.

Körtum and Seiler distinguish between "real" and "apparent" deviations from the law. An apparent deviation occurs when the absorbing material participates in an equilibrium in the solution, such as dissociation or formation of a complex. Many of the colored materials of interest in colorimetry have weakly acidic properties, and their equilibria are easily disturbed by variations in acidity. For this reason it is often necessary to control *p*H and other variables within narrow limits to obtain concordant results.

This is particularly true of the chromate-dichromate system which has served as a basis for permanent visual standards in a number of colorimetric methods, and also for checking the photometric values of spectrophotometers (see Chapter 5). Figure 2·4 shows the pronounced effect of *p*H on the chromate-dichromate equilibrium, in terms of transmittancy curves, and the necessity of buffering to a definite *p*H. Detailed recommendations concerning the use of chromate standards have been made by Kitson (*20*). The change in color resulting from dilution of a solution of the dichromate ion with water is a familiar example. Presumably the effect involves the equilibrium represented by the equation,

$$Cr_2O_7^{=} + H_2O \rightleftharpoons 2HCrO_4^{-} \rightleftharpoons 2H^+ + 2CrO_4^{=}$$

A real deviation from Beer's law occurs when the absorbing entities are mutually influenced as the concentration is increased. Interaction may occur also between the solvent and the absorbing entities, and its extent may be affected by the concentration. Nonabsorbing foreign substances can have a similar effect on the absorption, a phenomenon known as the "salt effect" in the case of indicators, but not limited to electrolytes.

As regards the effect of temperature on the light absorption of dissolved ions, the following effects have been observed (*24*). The bands are always displaced toward the red by an elevation in temperature. The effect is less the greater the state of saturation of the absorbing ion, that is, the less it can be deformed by external influences. If measured on the steep portion of the absorption curve, the shift will be shown by an increase or decrease in transmittancy, depending upon which side of the peak the measurement is taken. The magnitude of the change depends upon the material in question, the increase for chromate at 436 mμ being only 0.1 per cent per degree, compared with 1 per cent for the very sensitive 2,4-dinitrophenolate ion (*24*). Both these measurements were made on the steep portions of the curves where the shift in the band produces the greatest change in transmittancy.

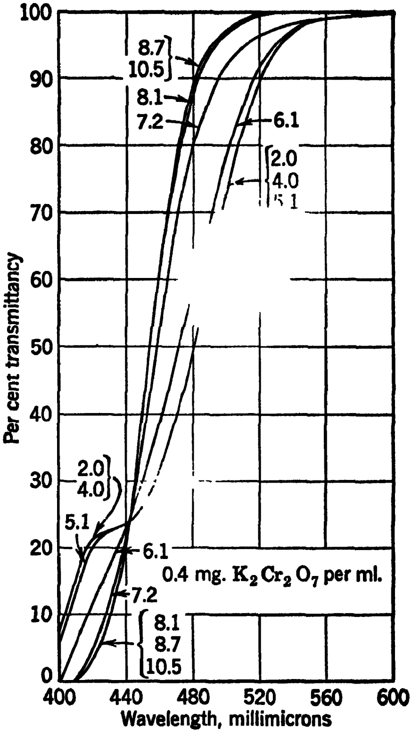

Fig. 2·4. Effect of *p*H on the transmittancy of a solution of potassium dichromate (*p*H given on the curves).

The concentration at which a perceptible divergence from Beer's law first occurs depends not only on the accuracy of the measurement but also on the material in question and the position of the absorption band. These may vary over a wide range. With some materials the divergence is so great that the region in which Beer's law is valid is not attained even at the lowest concentrations measurable. The absorbancy index of the methylene blue cation, for example, increases 88 per cent at 436 mμ in the range 10^{-5} to 10^{-2} *M*, and does not reach a constant value even below 10^{-6} *M* (*21*).

Sources of apparent and real deviations from Beer's law due to chemical causes may be summarized as follows:

1. Change in refractive index with concentration.

2. Displacement of equilibria involving the absorbing entities, such as ions.

3. Reciprocal interaction of the absorbing entities, either (*a*) among themselves, or (*b*) with the solvent or foreign substances.

From the analyst's viewpoint it makes little difference whether the deviation is real or apparent if he is unable to bring about conformity to the law by altering conditions over which he has control. Conformity to the law is desirable, as it usually indicates that satisfactory conditions are being observed, such as the proportions of various reagents used. An apparent deviation sometimes occurs when the constituent being determined exceeds the amount of reagent added to develop the color. The maximum color is not then obtained and the calibration curve may change slope very gradually at first, assuming the general appearance of a genuine deviation. Sufficient reagent, of course, produces the desired results in such cases. Validity of Beer's law must not be assumed, but should be checked in every case under the conditions of recommended procedure.

Hardy and Young (*16*) have discussed again the application of Beer's law from the standpoint of composition of the solution. Equations developed for a two-component system are supported by experimental work on a mixture of mutually nonreacting dyes.

2·15 Instrumental causes of deviation. What seems in a given case to be deviation from Beer's law may be the result of a low degree of monochromaticity of the radiant energy used. It has already been stated that the use of continuous sources, even with the best monochromators, gives a spectral band of appreciable width. Brattain, Rasmussen, and Cravath (*8*) have noted that such nonmonochromatic sources may cause apparent deviation if the transmittancy of the absorbing substance changes considerably across the spectral interval isolated by the monochromator. Particularly one may look for divergence in cases in which there is not a reasonably inverse symmetrical relationship between the transmittance curves of the solution and of the filter (*3*).

An apparent invalidity of the law may be observed in making measurements with the kind of heterogeneous light always obtained with simple glass filters. For spectrally impure light of the composition λ_0, λ_1, λ_2, $\cdots$, λ_n, the mean absorbancy index, a_s, is given by the various $(a_s)_0$, $(a_s)_1$, $(a_s)_2$, $\cdots$, $(a_s)_n$ values of the single component wavelengths of the light. The ratios of the intensities I_1/I_0, I_2/I_0, $\cdots$, I_n/I_0 change likewise. On passage of light through the absorbing layer, these ratios change, since the various components are absorbed to different extents. The mean absorbancy index, therefore, is dependent on the amount of light absorbed, that is, on the absorbancy and, at constant thickness, on the concentration. In testing Beer's law for divergences of the kind mentioned previously, this effect of impure light can be eliminated by working at a constant value of *bc*. Thus, with glass filters, a nonlinear calibration curve is not necessarily an indication of chemical deviation from Beer's law.

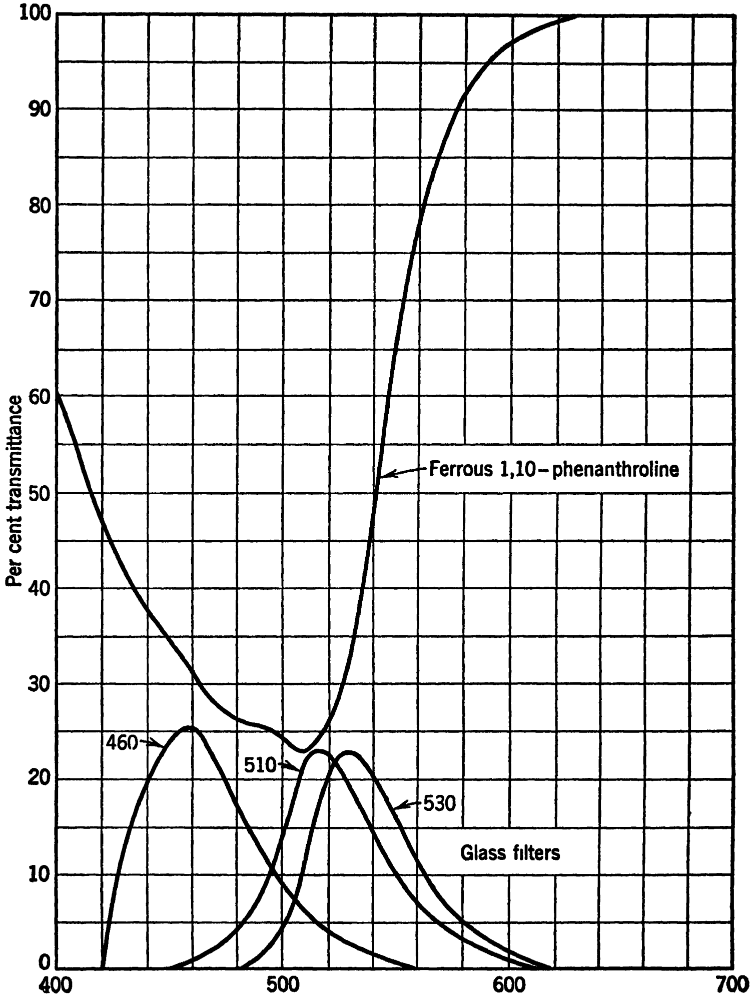

FIG. 2·5. Spectral transmittancy curve for the iron-phenanthroline complex; transmittance curves for three filters.

As an example of such a situation, Fig. 2·5 shows the transmittance curves for three filters, along with the transmittancy curve for the iron-phenanthroline complex. Then Fig. 2·6 shows the calibration curves for a photometer using each of these filters. In addition, there is included the calibration curve (broken, straight line) for the same solution

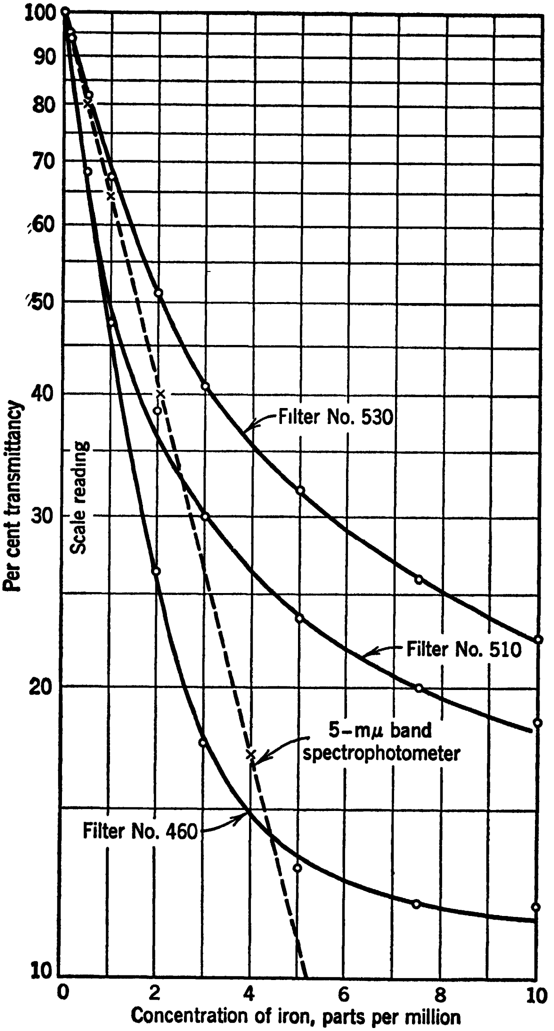

FIG. 2·6. Calibration curves for a filter photometer, using the three filters shown in Fig. 2·5.

using a spectrophotometer operating on a 5-mμ spectral band width. Incidentally these calibration curves are evidence for the writer's contention that the publication of such a curve for some instrument used in a particular investigation means little.

The most exacting case to meet instrumentally is the system having transmittancy curves with sharp minima, with the sides of the band unsymmetrical. Stray or scattered light in the beam transmitted may be a factor also.

E·DATA AND CONVENTIONS

If one has an instrument capable of yielding results of the desired accuracy, and if it has been adjusted and calibrated, there remains the question of how to handle the experimental data obtained.

2·16 General practice. There seems to be no single justifiable recommendation, even for plotting spectral absorption curves. With simple comparimeters, there is little to do except to record a match value for concentration. This is often true also for filter photometers and spectrophotometers, except that the value is for absorptivity, expressed in a suitable way. The marked differences to be found in the literature occur when it comes to plotting (1) calibration curves, such as those shown in Fig. 2·3, and (2) curves relating absorptivity to the spectral region measured. There may be found, of course, every possible variation in symbols and usages.

Although the terms *absorptimetric* and *absorptance* are used, more often the measurements are reflectance, R, for an opaque material, and transmittance, T_i (or transmittancy, T_s), for a transparent system. Reflectance is generally expressed as percentage. This basis is often used also for transmittance of solids and transmittancy of solutions. Hardy (*15*) and others prefer the decimal fraction, transmittance (or transmittancy) factor. Also there is extensive use of absorbancy, A_s, (formerly designated as optical density, D, or extinction, E), the absorbancy index, a_s, (formerly extinction coefficient), the molar absorbancy index, a_M, (formerly molecular extinction coefficient, K or ϵ), or the logarithm of one of these. A number of instruments read directly in terms of one or more such values. Occasionally the graduation of the photometer is in terms of a 90° sector angle or of a 45° polarization angle. Brode (*9*) gives tables for the interconversion of such values. Whatever the basis of the readings, they form the ordinates for curves. The region of the spectrum measured forms the abscissas, and the values are expressed in one or more of the following terms: wavelength, λ (in millimicrons, mμ, or angstroms, A), frequency, ν (in fresnels) wave number, ν' (in waves per centimeter), and logarithm of the wavelength. Wavelength, wave number, and frequency are related according to the expression,

$$\frac{1}{\text{Wavelength}} = \text{Wave number} = \frac{\text{Frequency}}{\text{Speed of light}}$$

the wavelength being m$\mu \times 10^{-7}$ cm., and the speed of light being 3×10^{10} cm./sec. Brode also gives tables for interconversion of these values. From the standpoint of the accuracy possible for the measurement, few graphs would seem to justify the significant figures implied in using angstroms.

Unfortunately, no one system of plotting has been adopted. In fact, different systems may be encountered in a single paper. This situation is the result partly of personal inertia and preference, and partly of the inadequacy at present of any one system for all requirements. With all possible variations in use, including inconsistency in the direction of plotting for any given combination, and disregard for good practice in graphing, the literature is likely to be less than clear to one unskilled in transforming mentally a curve in unfamiliar form into the form with which he customarily deals. Some advantages of several forms may be noted.

Transmittance (or transmittancy) is often the quantity obtained directly, especially with certain single-beam substitution or recording types of photoelectric instruments. Thus, presentation of the original data is easy. Since the light transmitted (reflected) determines the color, this kind of plotting is preferred by many in the designation of colors. One soon learns to associate curve form with hue. Also, curves in this form are the basis for the calculation of numerical color specifications, as noted in Chapter 9. Such a set of curves is shown in Fig. 2·7, with the abscissa scales given in wavelength, frequency, and wave number. Some writers plot log T_s on equal-division paper, or T_s directly on semilogarithmic paper. Either method greatly magnifies values below 10 per cent, in comparison to those above 70 per cent. Figure 2·10 shows the use of the semilogarithmic paper.

Absorbancy (optical density, or extinction to many) magnifies absorption maxima and thus makes them definite and easily read. Also it facilitates computation from one thickness to another, if desired, since the relation is linear for solutions conforming to Beer's law (see Fig. 2·9).

Curves of the percentage-wavelength, or absorbancy-wavelength, type often differ considerably in shape for different thicknesses of media or different concentrations of solutions. If one plots log A_s (log log $1/T_s$), the curves all have the same shape (*15*) regardless of thickness or concentration of solution (if it conforms to Beer's law). This is evident from the relation,

$$A_s = \log_{10} \frac{1}{T_s} = a_s b \tag{2·16}$$

Then,

$$\log_{10} A_s = \log_{10} a_s + \log_{10} b \tag{2·17}$$

Since the index, a_s, varies with the wavelength, and since the thickness (or concentration) of the sample, b, does not, the shape of the curve depends upon the term $\log_{10} a_s$, and the height upon the term $\log_{10} b$ (see Fig. 2·11). The absorbancy, A_s, may be plotted, of course, on a log scale paper.

In some laboratories this type of curve is used for standard curves and for the identification of substances. This practice is illustrated in Chapter 7. Curves of identical substances are superposable. In dyestuff manufacture and application, for example, the trade thinks in terms of shade and strength, variables immediately separable by this method as curve-shape and position variations, respectively. Shurcliff (*39*) proposed a curve-shape index for identifying dyes by means of such spectrophotometric curves. For reflectance data the ordinate is $\log (1 - R)^2/R$, in which R is the body reflectance (*1*).

In theoretical and interpretative studies on the relation of absorptivity and constitution, often including the ultraviolet and the infrared regions, frequency is of more fundamental importance than wavelength as abscissas, since it gives a better indication of the relative width of bands in the three regions. Shurcliff (*40*) preferred to use the logarithm of the wavelength (to the base 2), as shown in Fig. 2·12. The lower abscissa scale uses constant resolution units (C.R.U.).

As another point concerning data, mention may be made of uncertainty in definition and inconsistency in use of the terms employed. Thus, some curves labeled absorption are obviously either transmittance or transmittancy, but give no information to enable one to decide which. Frequently, also, there is inadequate information in publications for items such as the concentration of the solution, cell thickness, wavelength of the measurement, solvent, spectral band width, and temperature. To indicate all of these items (to the extent necessary), it may be desirable to have a statement such as

$$A_s \text{ (1 per cent, 1 cm., 425 m}\mu\text{, CHCl}_3\text{, 5 m}\mu\text{, 20°C.)} = 1800$$

The reader has a right to expect accuracy and clarity of presentation.

Finally, note may be made of what seems unjustified faith in published values of absorbancy or absorbancy indexes, including their use for plotting curves intended either for measuring amounts of constituents or for demonstrating the applicability of Beer's law to the system. Ideally, the absorbancy should be a physical constant comparable in reliability to other constants, such as refractive index. If the value is determined accurately, under carefully specified conditions, it may be so considered. But when one recalls that Beer's law presupposes monochromatic radiant energy and that few spectrophotometers, as generally used, come near meeting this requirement, the discrepancies are understandable. Often a point of doubt is the purity of the material measured. Perhaps this too implicit faith in absorbancy indexes accounts for many authors insisting on publishing absorbancy index-concentration graphs for their particular set of conditions. An experienced worker would not use the curve for another instrument in a careful analysis without checking.

2·17 Plotting data. Although a single determination or value, at some given wavelength, may suffice for a quantitative determination,

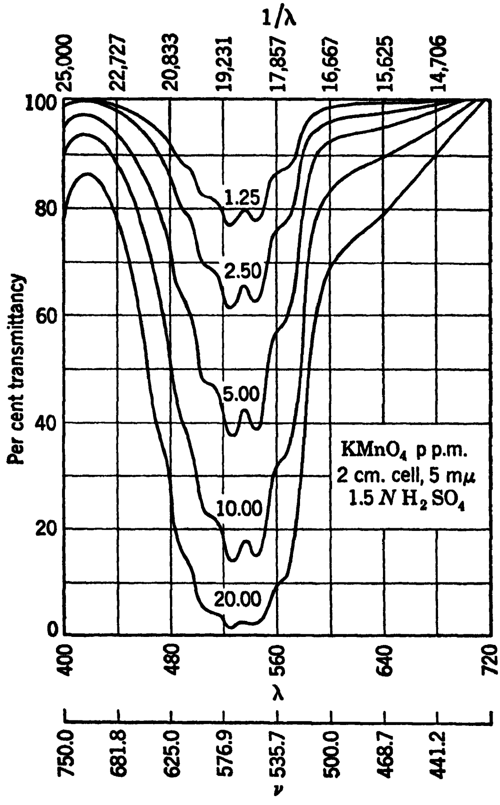

FIG. 2·7. Transmittancy-wavelength, transmittancy-frequency, and transmittancy-wave number curves for solutions of potassium permanganate (linear scales).

generally at least a portion of the region of the spectrum concerned is covered for either qualitative or quantitative determinations. The data are presented then in the form of tables or graphs showing the determined values of transmittance or reflectance. Figures 2·7 to 2·12 show

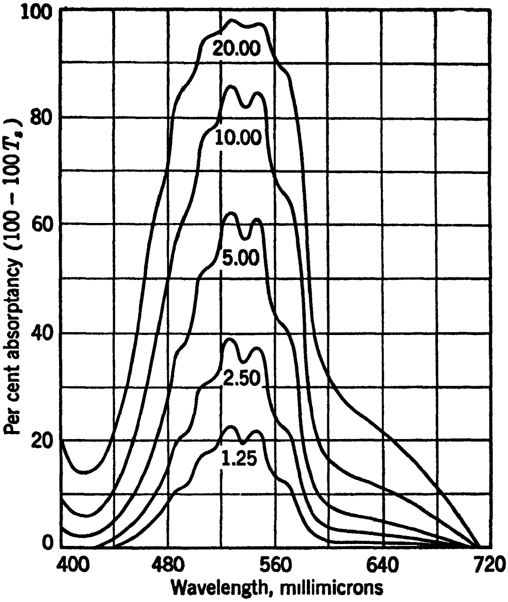

FIG. 2·8. Absorptancy-wavelength curves for solutions of potassium permanganate.

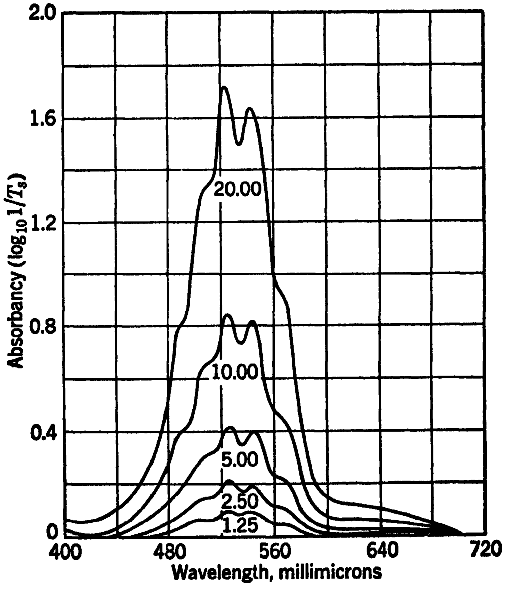

FIG. 2·9. Absorbancy-wavelength curves for solutions of potassium permanganate (linear scales).

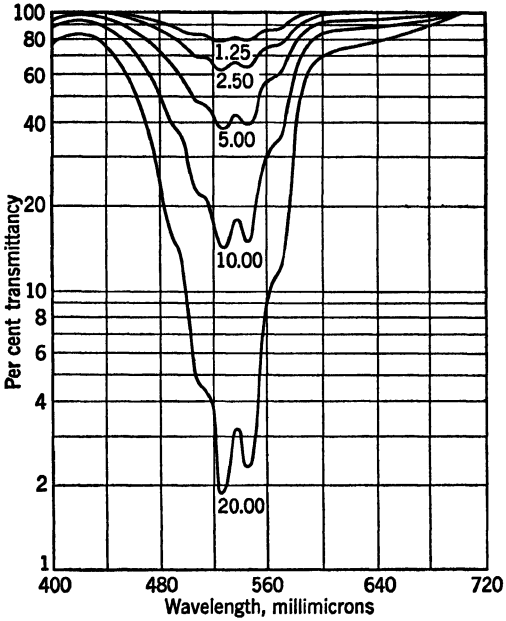

FIG. 2·10. Transmittancy-wavelength curves for solutions of potassium permanganate (semilogarithmic scales).

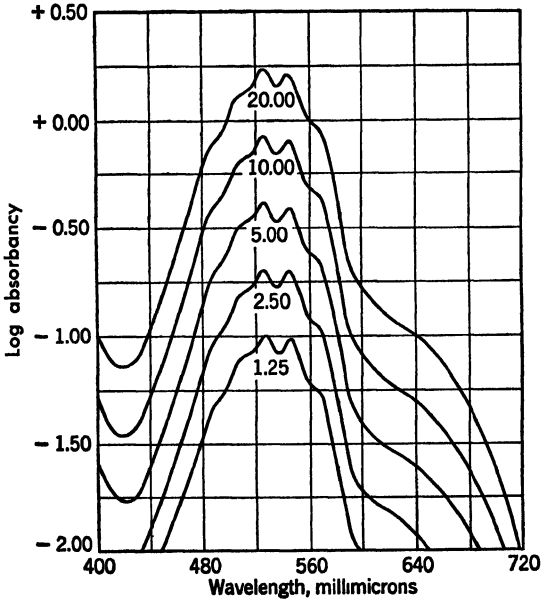

FIG. 2·11. Log absorbancy-wavelength curves for solutions of potassium permanganate (linear scales).

curves plotted in various ways for aqueous solutions of potassium permanganate. The curves in Fig. 2·7 came directly from a recording spectrophotometer, and the others are based upon these originals.* It should be noted that the transmittancy curves of Fig. 2·7 were obtained with solvent in one light beam and solution in the other, using matched

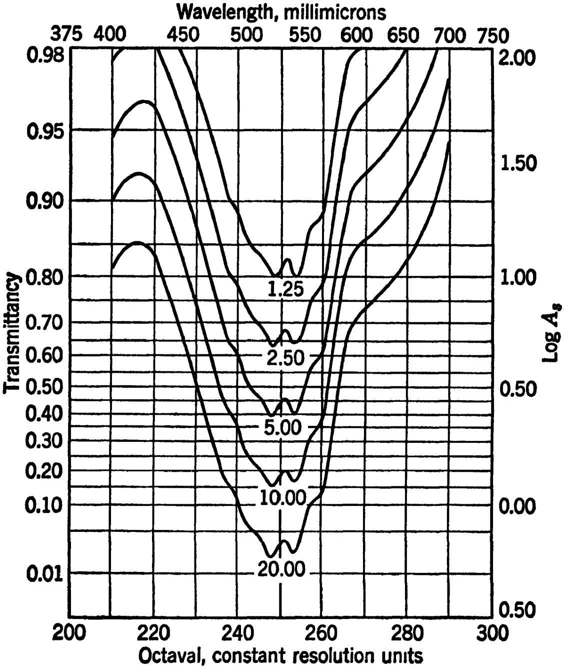

FIG. 2·12. Log absorbancy-log wavelength curves for solutions of potassium permanganate (linear scales). (Compare with plotting used in Chapter 7.)

absorption cells. Occasionally one sees a graph showing separately the transmittances of solvent and of solution for the same cell (*27*).

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* In plotting the data in Fig. 2·8 it was assumed that $1 - T = A$ (absorptancy). The danger of generalizing such an assumption is indicated in the quotation from the report of the Colorimetry Committee (*19*). "Computation of absorptance as unity minus the sum of reflectance and transmittance is incorrect in many cases because this quantity includes losses by internal reflection of light scattered in the medium, much of which is lost or absorbed at the edges of the object. Similarly, it is usually incorrect to consider the absorptance of an object observed by transmission as the difference between unity and the transmittance. This quantity includes the losses by reflection as well as the losses within the object resulting from both absorption and scattering. In the case of objects observed by reflection, the absorptance cannot be assumed to equal the reflectance subtracted from unity, for this difference includes the transmitted energy as well as the energy that is internally scattered and absorbed."

F·SENSITIVITY

In absorptimetry there seems to be no clearly defined and generally accepted means of expressing sensitivity comparable to milligrams/scale division, or scale divisions/milligram used for an analytical balance, or of milligrams for mass values measured with a balance. For work in the visible region Sandell (*38*) has defined sensitivity as "the smallest weight of a substance that can be detected in a column of solution having a unit cross section." From this viewpoint he discusses, for visual and photoelectric methods, the significance of two factors: (1) response of the receptor to small differences in transmittancy, and (2) the magnitude of the absorbancy index. Then sensitivities are tabulated for many of the common colorimetric methods for the metals.

Although this proposal for evaluation of sensitivity is probably the best, the literature contains various other suggestions. The reader should be aware of some of the most important of these.

2·18 Measures of sensitivity. Various units have been used to express sensitivity of color tests, including mass (*11*), mass per unit area viewed (*38*), concentration (*41*), molar absorbancy index (*3*), concentration required for a given transmittancy (*50*), and minimum detectable increase in concentration (*51*). Sensitivity expressed in mass units alone, such as micrograms, is inadequate to anyone unfamiliar with the details of the procedure, although this practice has been common. Mass per unit area is quite definite, as brought out by Sandell (*38*), since the depth of solution is immaterial if a given mass of colored constituent is observed. Concentration units, such as micrograms per milliliter, milligrams per liter, or parts per million, are usually preferred.

Molar absorbancy indexes are useful in comparing sensitivities of different reagents for the same constituent, although in comparing methods for constituents differing in atomic or molecular weight the absorbancy indexes may be misleading. For example, methods for beryllium (at. wt. 9) and for lead (at. wt. 207) giving the same instrument reading for equal concentrations of the metals would be said on this basis to have equal sensitivities, although, if calculated on the basis of molar absorbancy indexes, the method for lead would be 23 times as sensitive as that for beryllium.

To facilitate practical analytical work, especially the comparison of methods, Wernimont (*47*) has proposed using what may be called a specific absorbancy concentration, which may be defined as the concentration of solute, in parts per million or micrograms per milliliter, required to give the absorbancy a value of 1.00 for a 1-cm. cell. This eliminates the objection of using molar absorbancy index without regard to the actual weight of colored material involved.

Also for practical purposes, sensitivity in terms of concentration equivalent to a given value of transmittancy is a useful basis of comparison where the systems compared have similar hues. Woods (*50*) used this means of comparing sensitivities of various reagents for iron. On this basis, a light yellow might give greater sensitivity than a medium purple with monochromatic light, whereas the situation would be reversed with white light. To the unaided eye a yellow solution strongly absorbing in the blue region would not necessarily appear to be intensely colored.

The highest molar absorbancy indexes encountered in colorimetery have been in the neighborhood of 35,000. For example, nitrous acid, as determined by the Griess method, gives a value of 34,400 in comparison with 30,400 for dithizone and 35,600 for copper dithizonate (*28*).

In addition to the sensitivity of the color reaction, which is essentially chemical in nature, sensitivity of the measuring device is of considerable importance, as noted in other chapters in this book. Körtum (*23*), Ringbom (*35*, *36*), and others have studied this question in detail. Whether the measurement is made by visual or photoelectric means, the intensity and the spectral distribution of the light source are significant factors. Visual acuity and spectral sensitivity vary with the observer. With photoelectric instruments sensitivity is the product of several electrical factors, including output of the photocells for a given light intensity and response of the galvanometer or other measuring device to a given change in input. Then, too, sensitivity in spectrophotometers and filter photometers is a function of the spectral band width used (*49*). This is shown in Fig. 2·6, together with the effect of the location of the bands isolated by the filters used.

The over-all sensitivity of a colorimetric method, therefore, involves a number of chemical and instrumental factors, in addition to the human element if a visual comparison or measurement is involved. Over-all sensitivity, assuming adequate means of balancing the instrument reproducibly, is measured by the response of the instrument to a given change in concentration and is indicated by the slope of the calibration curve. The greater the rate of change of instrument reading with concentration, the greater is the over-all sensitivity of the method, both in detecting low concentrations and in distinguishing between concentrations having nearly the same value. With monochromatic light, maximum sensitivity is obtained at the wavelength of maximum absorption. This effect is shown in the Beer's law plots included in Fig. 2·3 for transmittancies at 525, 500, and 475 mμ. Reference to Fig. 2·7 shows 525 mμ as the wavelength of maximum absorptivity.

G·RANGE AND ACCURACY

In addition to the aspects of sensitivity discussed in Section 2·18, one is concerned in a general way with the amount of a constituent determinable absorptimetrically and with the probable reliability of the measurement. Because of the variables likely to be involved in specific cases, only broad statements have been attempted here.

2·19 Concentration range. If sensitivity were independent of the intensity of the transmitted light, it would be advantageous to work at high concentrations in order that a given error in concentration would be small percentagewise. Sensitivity falls off, however, at high concentrations. Extremely low concentrations also are not advisable, even though colorimetric methods are usually considered among the few kinds of procedures applicable especially to trace amounts of constituents.

The lower limit of measurable concentrations depends chemically only upon the sensitivity of the reaction and the purity of the reagents. In certain cases quantities as small as 10^{-6} mg. have been determined. Measurements on solutions containing 0.01 to 0.001 p.p.m. of various constituents are not uncommon practice. A general method of extending the lower range of a colorimetric method by adding to the sample a measured quantity of the desired constituent and then performing the determination as usual was described by LeRoy (*25*).

Since at high concentrations the change of color intensity with concentration is too small for accurate comparison by visual or other means, one usually resorts to dilution or use of smaller samples, provided the percentage error of the method can be tolerated with samples high in the constituent being determined. The principal use of colorimetric methods has been in measurement of small quantities, although current work is extending the application of colorimetry into the domain of the familiar titrimetric and gravimetric methods. For example, Putsche and Malooly (*34*) obtained satisfactory results on cobalt in stainless steel at concentrations exceeding 40 per cent. As much as 75 per cent of trinitrobenzene in dinitrobenzene is easily determinable with a relative error of 0.5 per cent (*32*). Mehlig has reported results comparable with good titrimetric work on samples such as copper and iron ores containing, respectively, more than 20 and 55 per cent of the metal (*29*). The relative error to be expected in much work, however, is of the order of 1 per cent, and frequently 5 per cent or more, especially with visual devices.

Curves such as those shown in Fig. 2·3 serve not only as calibration curves for use of an instrument, but also as an indication of the measurable range of the method for the cell thickness used. Likewise those in Fig. 2·7 show this range. Both representations of the data show that the range in this case is about 0.5 to 20 mg. of manganese per liter.

2·20 Optimum concentration. The question arises as to whether there is an optimum concentration, within this range, where a given observational error will give a minimum error in the calculated concentration. Only a general statement will be given here, since various factors are involved when one tries to cover visual, photographic, thermoelectric, and photoelectric methods, including different kinds of instruments in each class. Some consideration is given in Chapter 3 to visual comparimetric methods. Here the discussion is limited to filter photometric and spectrophotometric methods.

From the transmittancy-concentration curves of Fig. 2·3, it is obvious that a given change in transmittancy means a small change in concentration at low concentrations, and vice versa. For the 525-mμ transmittancy curve there is a range, between 5 and 10 p.p.m., of ratios of change of transmittancy to change of concentration intermediate between those near the ends of the curve. This occurs near 7 p.p.m. manganese (compare the conclusions reached later in connection with Fig. 2·13).

In contrast to the transmittancy-concentration curves, the logarithmic curves of Fig. 2·3 show a constant rate of change of the logarithmic value per unit change of concentration (for solutions conforming to Beer's law, of course). The curve for 525 mμ shows the maximum slope, because at this wavelength there is the greatest change in the logarithmic value per unit change of concentration. Because of this straight-line relationship, many seem to think that measurements are equally reliable anywhere within the measurable range of concentration.

In order to emphasize the importance of making measurements within certain transmittancy ranges, Ringbom (*35*) suggested plotting curves coordinating absorptancy (1 − transmittancy) on a linear scale, with concentration on a logarithmic scale. Later he and Sundman (*36*) applied the idea in the determination of copper, cobalt, and nickel. Use of this scheme is illustrated in Fig. 2·13 for three iron complexes and for solutions of potassium permanganate with readings taken at three different wavelengths. These are the same wavelengths used for the Beer's law curves of Fig. 2·3. The values for the permanganate solutions are taken from Fig. 2·7, and those for the iron solutions from the work of Fortune (*12*) and of Moss (*31*). Since transmittancy is used so much more in this country than absorptancy, curves for both values are included.

Recently Ayres (4) has directed attention again to this method of plotting data. The range in which measurement is possible is shown, of course, but with no apparent advantage in this respect over the transmittancy curves shown in Figs. 2·3 and 2·7. However, there is shown graphically, through this measurable range, the rate of change of transmittancy (or absorptancy) with unit change in concentration. Two regions should be noted: (1) that of small rate of change at very high or very low transmittancies; and (2) that of maximum rate at 28 per cent transmittancy (or 72 per cent absorptancy).

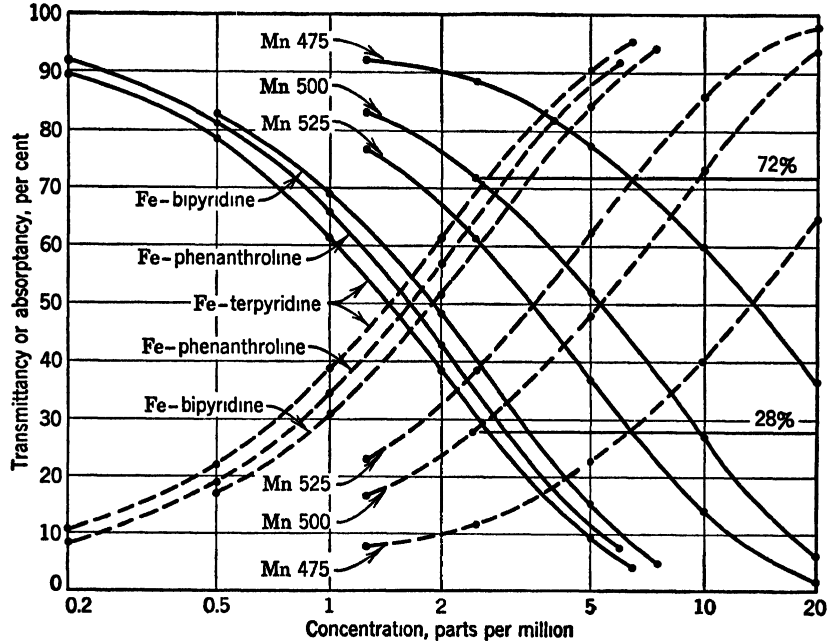

FIG. 2·13. Calibration curves for solutions of three iron complexes, and for solutions of potassium permanganate at three different wavelengths. Transmittancy——; absorptancy – – – – –.

For optimum photometric accuracy, therefore, one should have a system which will give a transmittancy of 28 per cent. Mathematical justification for this statement is given in Chapter 7, Section 7·27. Experimental control of conditions to make this transmittancy possible is limited to varying the concentration and the length of the absorption cell. The extent to which one may safely go above or below the optimum transmittancy depends upon the tolerance established.

It should be noted that the value of 28 per cent transmittancy is obtained mathematically in Section 7·27 on the basis of equations applicable to the Martens type photometer with electric balancing. This photometer is used in various instruments, including the General Electric spectrophotometer with which 'the data were obtained for Figs. 2·3 and 2·13.

In spite of widely accepted conclusions to the contrary, different optimum transmittancies apply to different types of instruments. Thus, Sandell (*38*) states that a transmittancy of 10 to 20 per cent gives the most precise results with visual spectrophotometers.

However, the commonly given values for transmittancy and absorptancy are, respectively, 36.8 and 63.2 per cent (*4*, *14*, *42*), with no qualification about the instrument. The writers concerned apparently have all assumed that the photometric error of analysis, as a function of the absorbancy of the solution, is given by the equation,

$$\frac{dc}{c} = -\frac{0.434}{A_s}\frac{dI}{I} \tag{2·18}$$

in which the symbols have their usual significance. On the basis of this equation of Ringbom and the assumption of an absolute error of 1 per cent in the transmittancy, Ayres (*4*) gives the following values for errors at definite transmittancies:

I, per cent	dc/c, per cent
60	3.3
50	2.9
40	2.7
30	2.8
20	3.2

In deriving the equations which gave a transmittancy of 36.8 per cent, Hamilton (*14*) assumed "that the galvanometer deflection is proportional to the intensity of light striking the photocell." His conclusions apply to the Beckman instrument and to others of this type.

In general, then, the table given by Ayres should be adjusted to bring the minimum error value opposite the transmittancy value appropriate to the instrument concerned. For the various types currently in use, the range will be approximately 10 to 60 per cent for the general magnitude of error given.

West (*48*) has contrasted subjective (visual) with objective (nonvisual) methods with respect to photometric accuracy and optimum concentration, sensitivity, and range.

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3· COLOR COMPARIMETERS

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A·GENERAL CONSIDERATIONS

3·1 Introduction. Among the classical methods used by chemists since the earliest history of analytical techniques, the determination of a desired constituent by producing a colored ion or compound and comparing the intensity of the resulting color with that of a known sample has received relatively little publicity. Even in the earliest days of chemistry semiquantitative determinations of constituents were made by viewing the colored solution and thereby estimating the amount of the constituent present in the light of past experiences with similarly colored solutions. A crude, although very common, current example is the practice of immersing a tea ball into a cup of hot water until the solution so formed attains the desired depth of color. With a little experience, most analysts today can estimate fairly closely the amount of permanganate in a dilute solution by looking at the color.

As the need arose for more accuracy, the early chemist discovered that he could make a better estimation of the amount of a colored constituent by comparing the solution with other colored solutions containing known amounts of the constituent. Thus, the standard series method of visual comparison evolved.

Many new techniques of visual comparison followed this discovery in rapid succession. Although many colorimetric methods were in use before that time, one of the first great advances in analytical colorimetry was made in 1854, when Jules Duboscq first designed and began the manufacture of the well-known comparator bearing his name (*9*). Since then many modifications and improvements have been made upon this original instrument, but the basic design has remained the same.

In its broadest sense, as discussed in Chapter 2, colorimetry includes all methods wherein the main objective is the determination of some constituent through the measurement of a systemic property related to the color of the system. The unknown system may be a gas, a liquid, or a solid; but normally an analytical colorimetric determination is made on a liquid system, usually in a solution of the unknown. It is possible to estimate colorimetrically certain gases, such as nitrogen dioxide, in a gaseous system, but such methods are few. Application of the comparator technique to solids is uncommon.

In the liquid systems, usually aqueous solutions, to which this chapter is generally limited, the desired constituent may be considered as the solute. The color formed in the solution which is to be compared with other standard solutions or solid standards generally will be either (*a*) inherent in the desired constituent itself, such as the permanganate ion or methylene blue molecule, or (*b*) produced by the reaction of the desired constituent with some color-forming reagents, such as is found in the determination of iron with 1,10-phenanthroline (*17*, *35*).

Generally, an analytical colorimetric method involves either the comparison of the colored solution with some standard colored solution or solid standard or the direct measurement of some colorimetric property of the system. This chapter is devoted to a discussion of the first of these two types of determination, namely, the comparison of the colored solution with a standard. The second type is discussed at length in later chapters.

As noted in Chapter 2, to the physicist the word *colorimetry* means the determination of color as color, or color specification. To the analytical chemist it has long meant the determination of the amount of a desired constituent by comparator methods of the kind discussed in this chapter. Now the analyst is making increasing use of the word *absorptimetry* to include those methods involving a determination of the absorptive capacity of a system for radiant energy. It seems legitimate to consider color-comparison procedures as relative absorption methods, since with them one compares the absorptive capacity of the system of unknown concentration with that of a system of known concentration of desired constituent (or with one visually equivalent).

3·2 Types of comparator methods. Once a colored solution has been prepared in which the intensity of the color is proportional to the concentration of the desired constituent, the methods of visual comparison * of this solution with a colorimetric standard lend themselves readily to classification into four general groups: standard series, duplication, dilution, and balancing.

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* Some instruments originally designed for use as visual comparators have been modified in an attempt to eliminate the visual factor. The modification of the Duboscq comparator by the addition of photocells to form a photoelectric comparator may be cited as an example.

Standard series methods depend upon comparing the intensity of color of the unknown solution with that of a series of standards, which consist of suitable liquid or solid materials. When the intensity of the color of the unknown solution matches the intensity of one of the standards in the series, the concentration of desired constituent in the unknown is equal to that represented by the standard. This type of method is probably the oldest, cheapest, and still the most widely used technique of comparison.

Duplication methods depend upon reproducing or "duplicating" the intensity of color in the unknown solution by adding known amounts of the desired constituent to another system containing the same reagents and the same final volume as the unknown. When a colorimetric match is obtained, the amount of the desired constituent in the unknown is equal to that added to the matching solution. This method has been called *colorimetric titration* by some authors.

Dilution methods depend upon securing a colorimetric match of the unknown solution with a known solution by diluting the more concentrated, known solution with solvent. Both solutions must be in comparison tubes of the same thickness and internal diameter and must be viewed horizontally. When a colorimetric match has been obtained, the concentrations are inversely proportional to the volumes of the solutions.

Balancing methods, coming more and more into general use, depend upon comparing the unknown solution with a single known solution, the depth through which one solution is viewed remaining constant and the depth through which the other is viewed being varied until a colorimetric match is obtained. Here, again, the concentrations of the known and unknown solutions, when colorimetrically matched, are inversely proportional to the depths through which they are viewed.

The technical aspects of the apparatus required for each of the above types of methods are discussed more fully later in this chapter.

3·3 General requirements for comparator methods. In Chapter 1 the chemistry involved in colorimetry has been discussed at length, including the general requirements for adapting a colorimetric method to a desired constituent. For the use of visual methods of color comparison certain of these requirements become paramount and warrant further discussion at this time.

It has been stated that the basic requirement for making a colorimetric determination is a colored solution. In order to use visual comparator methods, this colored solution must be easily comparable. Such a limiting requirement involves two factors: (*a*) preferably the color developed should be in that part of the spectrum to which the eye is most sensitive; and (*b*) the color should remain constant over a long enough period of time to permit at least three visual matches with the standard.

An examination of the relative luminosity curve (*23*) in Fig. 3·1 indicates that the human eye is best able to differentiate small changes in intensity of color in the wavelength region of 475 to 650 mμ. Colorimetric comparisons of deep blues or deep reds are often incapable of duplication within a range of less than 15 to 20 per cent, whereas a green or orange-colored solution may be compared visually with an error of less than 2 per cent. In the reddish purple 1,10-phenanthroline-iron complex the maximum absorptivity occurs at a wavelength of approximately 508 mμ. Since this point is not far from the region most sensitive to the eye, accurate determinations of iron can be made by using visual comparator methods. Aqueous permanganate, or diazotized 4-aminobenzenesulfonic acid coupled with 1-naphthylamine, is an even better example of more nearly true purple.

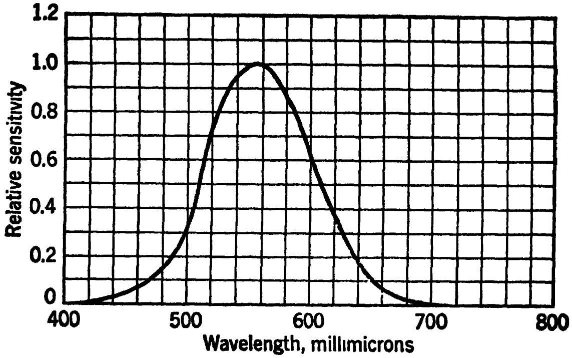

FIG. 3·1. Relative luminosity curve.

When visual color-comparison methods are used, it is preferable to make at least three matches and to use the average of the three values obtained. In order to permit the use of this matching technique, it is necessary that the colorimetric solution be stable for a period of time sufficient to permit the operator to make the necessary manipulations. Certain coupling reactions yield colored systems so instable that the indicating mechanism on a photoelectric instrument will "drift" across the dial during measurement. The reader can easily imagine the difficulty encountered when attempting to make visual comparisons of such fleeting colors. Although attempts have been made to use some of these reactions by taking readings at specified intervals after the addition of the color-forming reagent, generally visual comparison of these systems is unsatisfactory because of the time required to make the actual comparison. As noted in Chapter 1, a reasonably stable colored system is desirable for any kind of colorimetric determination, and it is almost imperative when visual comparison methods are used.

Another important requirement of a system for use in colorimetric methods is that there be no extraneous color inherent in the system or produced therein by interfering ions. This requirement also becomes imperative when visual comparator methods are to be used unless provision can be made for the interference. For example, in the determination of iron with 1,10-phenanthroline, the presence of nickelous or chromic ion changes the hue of the solution to be compared visually, in addition to forming complexes with the color reagent. Nitrites give a brown color to the solution at low *p*H values. When using filter-photometric or spectrophotometric methods, it is often possible to select a filter or a wavelength band such that inherent colors do not interfere with the measurement of the desired color; usually this is not feasible with visual comparator methods,* however, and all such interfering colors should be removed before the development of the colored system, unless compensation is possible. Another type of interference is the presence of ions which form complexes with the color-forming reagent. Such interferences are sometimes manifested by a fading effect such as may be seen when cobalt is present during the determination of iron with 2,2′,2″-terpyridine, or when cyanide ion is present in the determination of iron with 1,10-phenanthroline. As discussed under the stability of the colored system, accurate comparisons cannot be made visually during rapid fading.

A discussion of the principal requirements of a colorimetric system for use in visual comparator methods would be incomplete unless mention is made of the factors of speed and accuracy. In view of present-day requirements in the analytical laboratory for mass-production methods, it is often necessary to make an analysis of a material in a very limited time in order that changes in chemical composition may be effected during the processing of the material. Rapid analysis of steel samples may be required so that corrections can be made while the product is still in the furnace. In the production of organic products an excess of one of the manufacturing constituents may prove deleterious to the final material and must be corrected immediately upon receipt of the laboratory analysis. In certain biologicals, control of various by-products is extremely important and is accomplished through rapid analytical methods. Thus, it may be seen that a colorimetric method, to be feasible, must provide results with a minimum of preparation of the sample and a minimum of time for the development of the color and the reading of the final result. If the desired constituent could be determined directly by a titrimetric method, it would not be feasible to use a colorimetric method which involved the separation of the desired constituent before color formation and a long and involved process for developing the color. On the other hand, as will be discussed later, it would be very impractical to use a long, involved electrometric method of measuring the *p*H of a system when a rough estimate is required and could be obtained with one of the common *p*H test papers available. Since most colorimetric methods are chosen for analytical determinations because of the speed of the determination, this requirement is usually met without involved chemical manipulation.

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* Recently filters have been developed for the determination of certain constituents, using visual comparators. Usually these filters are placed in the optical system either directly above or below the colored solutions being compared.

The requirements of accuracy in a visual colorimetric method should be carefully considered. The operator can usually secure a value within 5 per cent of the amount present by visual matching, providing the other requirements of the colorimetric system are met. If closer accuracy is required, the colorimetric method may not be as suitable as certain other types of methods. The author has consistently secured results in the determination of iron with 1,10-phenanthroline within a range of 2 per cent of the amount present. Other research workers have claimed results on visual colorimetric methods varying not more than 1 per cent, although long experience was first necessary. Komar (*28*) reported an accuracy within 2 per cent in the estimation of various substances, using Hehner cylinders. Ringbom and Sundman (*33*) have reported good accuracy in certain visual comparator methods: thus, at a concentration of 0.3 gram per liter of copper in ammoniacal solution, the accuracy was in the range of 0.1 per cent absolute; cobalt in a concentration of 0.01 gram per liter as the thiocyanate blue complex has been determined with an accuracy of 0.6 per cent on a relative basis. Other examples are cited by these authors.

When only small samples are available, it may often be found that the results obtained by methods of visual comparison, requiring little preliminary treatment of the sample, are more accurate than those secured by methods involving lengthy chemical procedures. The clinical determination of thiocyanate in small amounts of blood can be performed with a higher degree of accuracy and in much less time by colorimetric methods than by more involved methods. A test kit is available to physicians, enabling them to make determinations of thiocyanate in a 1-ml. sample of blood in less than 5 minutes. The final comparison is made by a standard series method, using a color chart.

The accuracy is well within the limits required for the proper dosage of thiocyanate. Other kits are available for nearly every type of routine clinical testing involved in blood and urine chemistry.

3·4 Beer's law as applied to comparator methods. Chapter 2 includes a detailed discussion of Beer's law and the Bouguer-Beer relationships as applied to colored solutions in general. There it was pointed out that Beer's law ($T = t^c$) is valid for colored solutions only under certain conditions, such as the use of monochromatic light and the constancy of the nature of the absorbing ionic or molecular species at varying concentrations. Variations in association, dissociation, or ionization at different concentrations of the same solute in the colored system may produce deviation from Beer's law.

Unfortunately, many colored systems fail to meet these rigid specifications over a wide range of concentration, with consequent deviation from Beer's law. In the types of comparator methods wherein the visual color match is made under practically identical concentration and depth of solution, namely, *standard series* and *duplication types*, Beer's law need not hold, since the degree of association or other variable is practically the same in both standard and unknown through duplication in the physical preparation of the solutions. However, when *dilution* or *balancing types* of methods are used in making the visual match, the knowledge of, and correction for, deviation from Beer's law may be of high importance.

One may readily test for deviation from Beer's law with a Duboscq balancing type of instrument (Section 3·17). The standard may be placed in one cup, and successive dilutions of the standard in the other cup. If the actual readings are identical with the calculated readings, it may be assumed that the system obeys Beer's law, and no correction need be applied. If, however, this is not experimentally true, some basis of correction must be established for the system being compared, unless the error may be disregarded.

For routine work the preparation of a correction curve for use with a colored system showing deviation from Beer's law is relatively simple. One need only prepare a series of standard solutions for the system being analyzed and, using one of the medium concentrations in the standard cup of the comparator, read the settings of the comparator when the other standard dilutions are placed successively in the unknown cup. The readings on the unknown cup are plotted against the concentrations. An example of such a correction curve is shown in Fig. 3·2.

A second way of preparing such data for routine use consists in plotting deviation or error against observed values. It should be noted that for solutions more concentrated than the standard the correction will be negative, whereas for solutions more dilute the correction will be positive. Figure 3·3 illustrates such a method for plotting deviation from Beer's law.

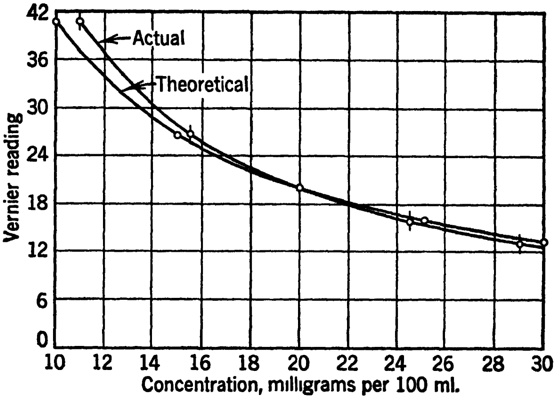

Fig. 3·2. Color comparator correction curve for deviation from Beer's law.

There are other ways of preparing correction curves, but the methods described are generally used in the analytical laboratory (*42*).

For very precise work, it is recommended that the calibration curves be prepared from a number of readings covering the dilution range in

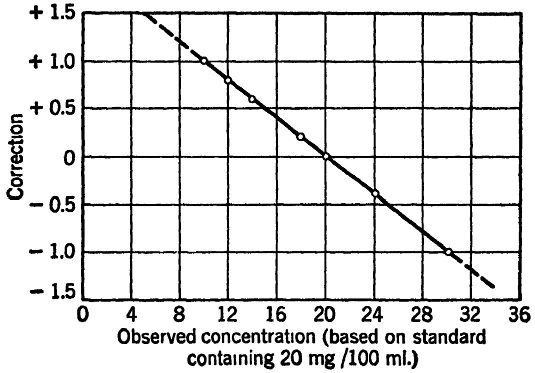

Fig. 3·3. Color comparator correction curve for deviation from Beer's law.

small increments. Such curves correct for deviations from Beer's law, and also compensate for subjective as well as equipment error encountered in the specific method.

3·5 Illumination for color comparison. Color comparison as carried out by the chemist consists of matching two bands of visible rays not absorbed by the standard and the unknown. If the standards happen to be of a composition other than that of the unknown, that is, artificial liquid or glass standards, then certain limiting conditions may need to be imposed on the source and type of illumination used for viewing the systems.

Since artificial standards are prepared by comparison with known solutions under specific conditions of illumination, it can readily be seen that these same specific conditions should prevail when the permanent standard is compared with an unknown system, unless the spectrophotometric curves of the two systems agree closely (*13a*).

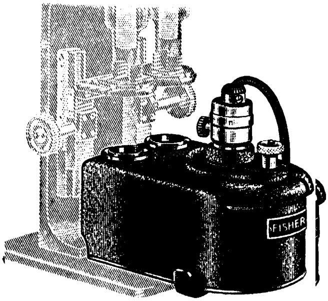

FIG. 3·4. Comparator illuminator. (Courtesy of Fisher Scientific Company.)

The ideal condition of illumination in making a visual comparison would be the use of a standard diffuse sunlight. Priest (*31*) established the theoretically most accurate "sunlight" as the radiant energy from a gas-filled standard tungsten lamp operating at a color temperature of 2848°K. The light from this lamp must be passed through a pair of crossed nicol prisms containing between them a quartz plate 0.500 mm. thick with surfaces perpendicular to the optic axis of the plate.

Such a source is impractical for routine comparisons. However, light approximating this ideal condition can be secured by using certain gelatin or glass filters, such as Wratten No. 78. Certain Mazda lamps also have been made to approximate this ideal.

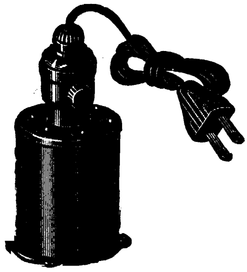

FIG. 3·5. Comparator illuminator. (Courtesy of Hellige, Inc.)

Some commercial instruments are designed to produce standard light for comparison purposes. The illuminator * shown in Fig. 3·4 can be attached directly to the base of many types of balancing instruments. This device provides two degrees of light intensity by the use of a diaphragm which may be inserted in the housing. When the diaphragm is moved into place, the light intensity is diminished to one-half with no change in color. The interior of the case is painted with dull white lacquer, and a reflector behind the bulb concentrates the light at the end of the case containing the windows. The light is reflected directly into the color comparator.

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* The specific commercial instruments mentioned and illustrated throughout this chapter are intended to be illustrative of a class of instruments rather than as necessarily the best of the specific type.

A second type of illuminator, shown in Fig. 3·5, is designed for use with roulette disc comparators containing standard glasses where direct illumination is desirable. A glass filter plate provides an illumination approximating normal daylight from a 110-volt tungsten lamp. Provision is made for attaching this illuminator directly to the comparator.

3·6 Errors in comparator methods. In the consideration of the application of a colorimetric method to a specific analytical problem, a careful examination of errors likely to be encountered and the sources of these errors is important. Often one type of method may involve fewer possible sources of error or more easily correctible errors for a specific determination than other types and, consequently, may prove more adaptable to the problems at hand. Likewise, in the selection of an instrument for colorimetric work, a knowledge of the sources of error likely to be encountered may indicate, for example, that a micro type instrument would be superior to a macro type instrument.

Comparator methods are subject to error in the preparation of the solution of the unknown and of the solution of the standard (if one is used) and in color matching. Errors likely to be encountered may be divided into three general classes:

a. Mechanical or equipment errors: errors due to faulty manufacture or improper care and treatment of the instruments used.

b. Systemic errors: errors due to specific characteristics of the colored solutions being compared.

c. Operational errors: subjective errors due to physical disabilities, insufficient training, or carelessness on the part of the technician making the comparison.

3·7 Mechanical or equipment errors. When a new instrument is received from a manufacturer, it has usually been carefully checked before shipment. It is not, however, good practice to assume that such an instrument is in perfect condition without making some tests. Jarring during shipping and force or tension during packing or unpacking may appreciably change some adjustment. Dust may find its way into the optical system after factory inspection. The plungers on a Duboscq type comparator, for example, may be forced out of plane with the optical system. The vernier may be slightly damaged. Thus, it is obvious that some check should be made to determine that the new instrument is mechanically in good condition before use.

Likewise, an analyst should always be aware of the possibility of some damage having been incurred, since the last use of a comparator or similar instrument, as a result of careless handling or cleaning. Fortunate, indeed, is the analyst who has exclusive use of, and responsibility for, the instrument on which he makes his comparisons. If the instrument has been handled by any other person before a determination, it is always well routinely to check the calibration.

Such a check is relatively simple to make. With a Duboscq type instrument note that the cups have not been interchanged, if error may arise from variation in the thickness of the bottom of the cups. Place standard solution in both cups. Proper readings will then indicate proper calibration. If incorrect readings are obtained, check the optical system for dust or for residue from previous solutions. Note that the plungers are properly and tightly seated. If the optical system is clean and apparently in satisfactory condition, then probably the vernier scale has become loosened and has changed its position. Adjustment can be made accordingly.

Another source of error which the author chooses to call equipment error has been discussed by Dehn (*12*) under the title "Fallacies in Colorimetry." Snell and Snell (*36*) review Dehn's work as "Optical Errors of the Solutions." It is believed, however, that the possible errors discussed below lie in the design of the equipment and in the use of this equipment and the optical systems, and hence fall into the category of equipment errors.

In most adaptations of balancing colorimetric methods the technician attempts to match specific depths and concentrations of the standard with varying depths and concentrations of the unknown solution. Usually both depth and concentration of the unknown are different from those of the standard. When such a match is obtained, the assumption is made that the solute molecules are identical both in number and in composition. Studies to disprove this limiting condition have been made on solute molecules in general with respect to (1) their different optical distributions in the solution, (2) different equilibria resulting from ionization, (3) different equilibria resulting from varying degrees of hydrolysis, and (4) different equilibria resulting from chromoisomerization. Since the last three studies are specifically errors of the colored system itself, only the first will be discussed here.

In studies on optical distribution of the solute molecules in colored systems three variables must be considered, volume, depth, and cross section. When viewing two solutions in a color comparator, for the two solutions to appear exactly identical they must be viewed at equal distances and the lines of the major axes must intersect at a point on or slightly behind the retina. Although the human eye presents a measurable area of vision, it actually operates as a point and permits compensation for the above requirement. Furthermore, since error would be introduced because of the apparent difference in size (truncated cone effect) of the upper and lower planes of the cylinder in which the solutions are viewed, the best modern instruments closely juxtapose portions of the centers of the fields wherein the concentric rings of top and bottom planes of the cylinders merge and thus, for practical purposes, eliminate such error. These instruments also provide for viewing the fields at a considerable distance from the cylinders. Thus, if the concentrations of the solutions being matched are not widely different, these errors become negligible.

Likewise, reasonably close concentrations of the two solutions eliminate any error due to a much larger number of colored solute molecules or ions being viewed in one cup than in the other, with a resultant greater absorptance of light and a less apparent smaller size of the more distant active solute species in the greater depth.

The technician should note also that error may be introduced through the use of depths of solution too shallow for comparison purposes. An ideal comparison container would consist of a hollow sphere with the point of comparison coincident with the center of the sphere. This requirement is met in most instruments by the use of fairly long comparison cups. If, however, a shallow solution is compared in one cup, due to too high a concentration of the solute ions or molecules, it may be noted that the observed color tends to become darker in shade nearer the edge, and a positive match is very difficult to obtain. Again, the use of nearly equal concentrations of standard and unknown almost entirely eliminates this source of error.

Where there are widely variant concentrations, and proper dilutions cannot be made (extremely rare conditions), Dehn (*12*) has proposed an equation to evaluate the degree of error involved:

a = distance from nearer surface
$a + b$ = distance from farther surface
r = radius of nearer surface
c = distance nearer surface is moved toward farther surface

The ratio of the viewed volumes is expressed as

$$1 : \frac{a^2[(a+b)^3 - (a+c)^3]}{(a+c)^2[(a+b)^3 - a^3]}$$

instead of $1 : \frac{b-c}{b}$.

If, for example, $a = 2$, $b = 2$, and $c = 1$, then the ratio of volumes becomes 1:0.29 instead of 1:0.50, an error of 42 per cent. If the distance is much greater and $a = 10$, $b = 2$, and $c = 1$, then the ratio becomes 1:0.45 instead of 1:0.50, an error of only 10 per cent, only twice the limitation of the usual visual colorimetric method.

By a careful selection of the type of instrument most readily adapted to the analytical problem, the analyst may eliminate nearly all the sources of error attributable to the equipment. By the proper use of this instrument, the remainder of the error encountered may be minimized.

3·8 Systemic errors. As previously indicated, these errors are due to specific characteristics or compositions of the solutions being compared. They are inherent in the colored solution or standards and must be eliminated during the preparation of the desired constituent for comparison. The general aspects of this problem are covered in Chapter 1. Certain of these systemic errors become of paramount importance in visual comparison methods and must be carefully avoided or corrected in designing an analytical procedure.

a. Interfering ions are of importance in any colorimetric method and particularly so in comparator methods, where a slight change in hue of the unknown solution from that of the standard may make matching impossible. Unless a reagent is specific for the constituent being determined, the presence of other ions in the solution may cause the formation of either more or less color of the same hue or may change the hue entirely. In the determination of iron with 1,10-phenanthroline (*17*) a study was made of fifty-five selected ions for interference. It was necessary to remove ions causing interference before the forming of the color. In many other colorimetric determinations published in recent years (*29*, *30*, *38*, *41*) much time and work have gone into the study of interfering ions. Normally it is preferable to form complexes or to remove such interfering ions before developing the color; but if separation is not feasible, there are certain corrections which may be applied for the interference.

When solutions have a natural color, such as may be exhibited by some biologicals like urine, it may be possible to add the same color to the standard itself. It may even be possible to superimpose the colored unknown over the standard itself. This principle may be used in the simple block, or more refined Leitz two-stage, comparator.

Another corrective measure sometimes used consists of determining by a spectrophotometer the wavelength of the point of greatest change in transmittancy of a solution with change in concentration. A filter, such as glass, gelatin, or liquid, can then be selected to pass light only in the wavelength region of maximum change of transmittancy. The filter can be used either over the eyepiece of the comparator or in front of the light source. The transmittancy of some interfering colors may thus be reduced practically to zero, and a colorimetric match can be obtained.

Control of *p*H often is of major importance in an analytical method depending upon the nature of the colored system and the other ions present. In the determination of iron with 1,10-phenanthroline, mentioned previously, *p*H could be varied from 2.0 to 9.0 without appreciably changing the color of the complex. Such is not true, however, in the determination of chromium as the chromate, where the *p*H must remain above 5.0. At *p*H values much below 5.0 some of the chromate ion changes to the dichromate, with a resultant change in the hue of the solutions. In any colored solution containing a *p*H indicator, small changes in *p*H may produce widely variant hues and so complicate color matching (*14*). Figure 3·6 shows spectrophotometric curves for methyl orange at various *p*H values (*18*). A solution of *p*H 2.6, curve 1, is reddish purple, while a solution of *p*H 4.98, curve 13, is yellow. It is relatively easy to determine the effect of *p*H on the colored solution by using two standard solutions in the comparator, obtaining a match, and then changing the *p*H in one of them. If the match remains un-

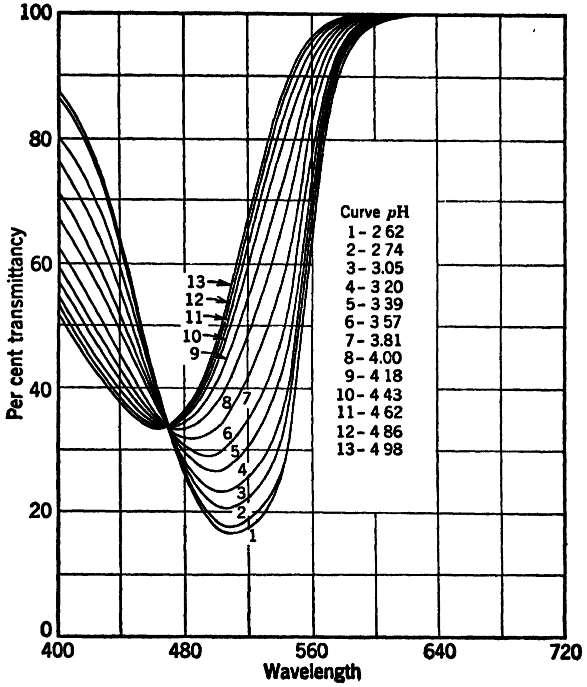


FIG. 3·6. Transmittancy curves for methyl orange solutions at different *p*H values. [Fortune and Mellon, *J. Am. Chem. Soc.*, **60**, 2607 (1938).]

changed at the new *p*H, then further change of *p*H can be made until an upper and a lower limiting *p*H, if there are such, are obtained.

When other ions are present, the control of *p*H may become of paramount importance for maintaining matching hues. In the determination of iron with 1,10-phenanthroline, for example, small amounts of copper may be tolerated if the *p*H is controlled between 2.5 and 4.0. Likewise, beryllium ion does not interfere at concentrations up to 50 p.p.m. when the *p*H is held between 3.0 and 5.5. Above *p*H 5.5 the hydroxide precipitates, and below *p*H 3.0 a complex is formed between the beryllium ion and 1,10-phenanthroline. Similar control of interferences by *p*H adjustment may be found in many other colorimetric methods. In some methods using dithizone, actual chemical separations may be made by varying *p*H. Kolthoff and Sandell (*27*) have described many color formations, using this reagent under carefully controlled *p*H conditions.

b. Dilution errors may enter into any analytical method wherein the initial concentration must be reduced before making the actual measurement of the desired constituents. One of the advantages of most colorimetric methods lies in the small amount of desired constituent needed for the determination. In the analysis of a potable water for iron, usually no dilution of the sample is necessary and the determination can be made directly. Often, however, the desired constituent may be present in many times the maximum quantity permitted in the color reaction, and dilution must be made. If the dilution is only a few-fold, the normal error encountered in a delivery pipet is sufficiently low that the total error will not exceed the error normally expected in a comparison method. On the contrary, in a colorimetric method for determining one of the new antibiotics (*37*) the dilution error on an absolute basis may appear very large. With a sample containing 100,000 μg. of the antibiotic per milliliter of solution, it is necessary to dilute until the final colored solution contains not more than 100 μg. per milliliter. This involves a dilution factor of 1000. It may readily be seen how a very small error in pipets or volumetric flasks may multiply the absolute error by a very large factor. However, it should be noted that percentage error does not appreciably change.

Dilution of artificial standards should usually be avoided as far as possible unless it is known that all components of the standards obey Beer's law. Usually solutions of two or more colors are combined to form the artificial standard. If one of the colors changes relatively more than the others on dilution, visual comparison of the diluted standard with an unknown will be impossible because of the change of hue of the standard. If it is possible to choose a sample weight such that the concentration of the desired constituent is very near that of the standard without dilution, the source of error described may be ignored for routine determinations.

c. *Reagent errors* may arise in any colorimetric method in two ways: first, errors caused by impure reagents; and second, errors caused by varying concentrations or amounts of added reagents.

Reagents classified as chemically pure (*C.P.*) cannot be assumed to be free from interfering impurities unless a "blank" test is made. In making such a "blank" determination, all the necessary reagents are added to a sample of carefully distilled water. The presence of color in the "blank" solution will indicate interfering impurities. The author has encountered interfering amounts of iron in "C.P." hydrochloric acid and in "C.P." hydroxylamine. Undoubtedly, any careful analyst can cite various examples of impurities in reagents. Fortunately, with the introduction of analytical reagent grade (*A.R.*) chemicals most of such sources of error have been removed, and some chemical houses now produce specially purified reagents for specific determinations.

The problem of concentration of reagents, or of the amount of reagent to be added in an analytical method, is usually solved by the chemist who originally devises the method. Very often the concentration of one or more of the reagents used to convert the desired constituent to a quantitative colored system is quite critical. In the determination of blood sugar by the use of 2,4-dinitro-1-naphthol-7-sulfonic acid (*16*, *36*) a variation of 1 per cent in the amount of trioxymethylene added causes an error of 10 per cent in the final analysis. In some colorimetric methods the desired constituent must be oxidized or reduced before determination. Insufficient oxidant or reductant in these methods causes serious error. In other methods the addition of a standardized amount of color-forming reagent is directed. The addition of appreciably more reagent may form complexes invalidating the accuracy of the results. If care is used in the preparation and use of the reagents in a colorimetric method, these sources of error may be entirely eliminated.

d. *Dichroism* may present a serious source of error in determinations using certain types of indicators. Figure 3·7 shows the spectral transmittancy curves for bromocresol green, an indicator used in *p*H determinations. It may be noted from these curves that the two absorption bands change at an unequal rate with change of *p*H.

Studies on dichroism (*14*, *7*) have indicated that solutions showing the phenomenon of two unequally changing absorption bands exhibit a different hue in thick layers than in thin layers, or in different concentrations at the same thickness. Bromocresol green at *p*H 4.5 appears reddish in 10-cm. thickness and greenish in 1-cm. thickness.

Errors caused by dichroism may appear in artificial standards. If dichroic solutions cannot be avoided in the preparation of artificial standards, such errors may be minimized by arbitrarily choosing concentrations or thicknesses of comparison solutions appearing to be intermediate in intensity of color between the high and low concentrations of the standard.

e. Turbidity and colloid errors usually do not enter into a determination if the colorimetric method has been carefully worked out and if all reagents are added in the proper order. Many of the known colorimetric methods can be made to show colloidal particles or turbidity by addition of large excesses of reagents. Turbidity in a colored solution makes comparison difficult by changing the "brightness" of the solution. The addition of the gray in the comparator tube containing the turbidity caused by the fine precipitate diminishing the light from the illuminant makes comparison difficult if not impossible.

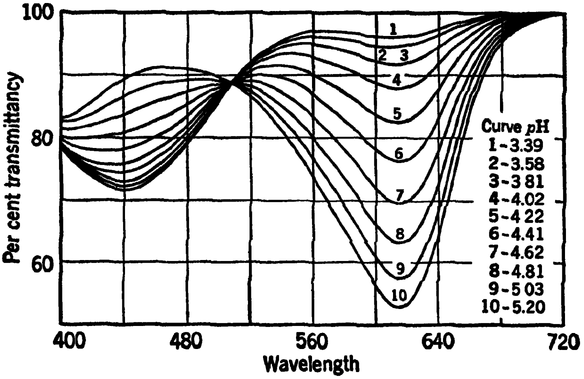


FIG. 3·7. Transmittancy curves for bromocresol green solutions at different *p*H values. [Fortune and Mellon, *J. Am. Chem. Soc.*, **60**, 2607 (1938).]

f. Temperature errors may occur occasionally. In some methods, such as certain modifications of the molybdenum-blue reaction, heat is required to produce the color. If the technician is not careful in following details, errors may arise because of incomplete color development. Some colored solutions vary in color intensity with change in temperature after the color has been completely formed. It is well known that cobaltous chloride in water solution appears reddish purple at 25° and blue at 100°C.

3·9 Operational errors. As previously stated, this class of errors includes subjective errors due to physical disabilities, insufficient training, or carelessness on the part of the technician making the comparison.

It is a well-known fact that certain colors can be matched more readily than others. Greens are much less tiring to the eye than deep blues and deep reds. A technician making a great many comparisons during the day may experience eye fatigue and consequently may have more and more difficulty in securing a colorimetric match as the day progresses. Such error may readily be avoided by changing technicians at intervals during the day or by dividing the comparisons among various operators.

Much controversy has arisen in the past as to the ability of a color-blind person to make color comparisons. Normally such a person is color blind to only a small portion of the spectrum and may even be more sensitive than average to the remainder of the spectrum. Colored solutions to which one technician is color blind may be referred to another technician. The author worked with a technician who was color blind to all parts of the spectrum but who was able to make satisfactory comparisons of certain colored solutions by matching "grays," since all colors appeared to him to be of the same hue but varied in brightness (see Chapter 9). This individual had great difficulty, however, in comparing unknowns with artificial standards composed of two or more colored components.

Variation in illumination due to carelessness of a technician, or even variations in sky color from a cloudy, dull day to a bright sunshine, may lead to error. As discussed earlier, many artificial standards are intended for use under a specific type of light, and variation may introduce error. The consistent use of a suitable commercial illuminant will eliminate such error.

B·TECHNIQUES OF COMPARISON

3·10 Standards. It was noted in Section 3·6*b* that one source of error may arise in unreliable standards. Since the kind of measurement covered in this chapter rests upon comparison of the system of unknown concentration with some standard, obviously the characteristics of this standard are important. Some of the principal points of concern are considered briefly.

Distinction is generally made between nonpermanent and permanent standards, although there is no definite dividing line. A permanent standard should be usable for several weeks, and ideally for an indefinite time. A nonpermanent standard may be so instable that it must be prepared at the same time, and under the same conditions, as the solution of the unknown.

Account should be taken of the chemical composition of the system containing the unknown and of the standard system. When they are the same, the standard may be considered a *self-standard.* In contrast, an *artificial standard* has a composition different from that of the unknown system.

As noted in Section 3·5, the conditions of illumination may be of great importance in visual comparisons. What one sees depends especially upon the spectral distribution of the illuminant and upon the nature of the absorption in the object observed. Preferably, therefore, the standard should have the same composition (light-absorptive capacity) as the unknown in order to avoid possible differences in the quality of the light reaching the eye. Adoption of artificial standards having a composition different from that of the unknown comes about usually because of greater convenience in the use of the standard, or of greater ease in its preparation. Examples of each type are cited later.

3·11 Liquids. Although recent years have brought an extension of the possibilities of quantitative determinations by selective reflection of light from essentially solid surfaces, the great majority of analytical comparimetric measurements is made on solutions.

For the preparation of a standard solution of the desired constituent, whether self-colored or to be used with a color-former, it is most desirable to have a primary standard, such as potassium dichromate, which may be weighed directly. Other materials, such as "Analytical Reagent" iron wire, or ferrous ammonium sulfate hexahydrate, may have sufficient purity to warrant disregard of the error for the measurements intended. The alternative to this approach, as in general titrimetry, is to standardize an approximately prepared solution. An example is the use of either anhydrous or hydrated ferric chloride. However made, such a solution, if stable, is then ready for making up working standards of the desired concentrations. If desirable or necessary, the color may be developed in these solutions simultaneously with that of the unknown, and the comparison made under identical conditions. In some methods, as the determination of iron with 1,10-phenanthroline, the developed color is stable for months, so that the standards may be sealed in tubes or bottles for later use. If such permanent self-standards are not feasible, self-standards may have to be prepared as often as each time a determination is made.

It may be physically impossible or inconvenient to prepare self-standards. For example, in the determination of residual chlorine in water by the *o*-tolidine method (*13*), it is very difficult to prepare a standard solution of chlorine water, and the solution is not stable for any reasonable time. In such methods it is necessary to resort to artificial standards.

A useful general series of artificial liquid standards was devised by H. V. Arny and his students. Originally proposed for caramel and tincture of cudbear, they were later adapted to wider use (*2*, *3*, *4*). These standards are based on three separate series, one containing a 0.250 *M* solution of cobaltous chloride, a 0.167 *M* solution of ferric chloride, and a 0.250 *M* solution of cupric sulfate in 1 per cent hydrochloric acid; the second consisting of solutions of 0.0100 *M* cobaltous chloride, 0.00167 *M* ammonium dichromate, and 0.0200 *M* cupric sulfate in 2.8 per cent ammonium hydroxide; and a third consisting of neutral solutions of 0.000200 *M* potassium permanganate and 0.00167 *M* potassium dichromate. Various proportions of the solutions within a series

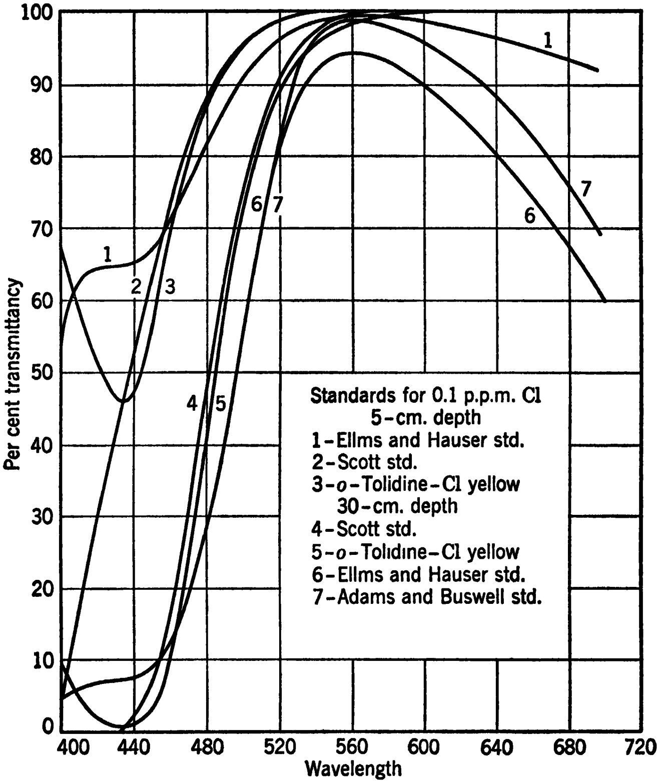


FIG. 3·8. Transmittancy curves for proposed permanent standards for the determination of residual chlorine by the *o*-tolidine method. [Dragt and Mellon, *Ind. Eng. Chem., Anal. Ed.*, **10**, 256 (1938).]

can be mixed to provide almost any color desired except the deep reds and blues. Spectrophotometric studies have shown the general colorimetric properties of these solution mixtures as artificial standards (*25*). It should be noted, however, that the ammoniacal series is not stable indefinitely.

Mixtures of potassium chloroplatinate and cobaltous chloride have been specified by the American Public Health Association as artificial standards for the determination of iron by the thiocyanate method (*1*).

A spectrophotometric study of the liquid artificial permanent standards proposed by various authors for the determination of residual chlorine by the *o*-tolidine method has been published by Dragt and Mellon (*13*). The spectrophotometric curves for the *o*-tolidine-chlorine yellow and for three proposed artificial standards at two different depths of solution are shown in Fig. 3·8. An examination of these curves indicates that the potassium chromate-dichromate mixture as proposed by Scott (*34*), buffered at *p*H 6.5, most closely approximates the curve of the known standard. The marked disagreement between the known standard and another proposed artificial standard is indicated in curves 1 and 3. The Scott standard was found to be colorimetrically more nearly a match for the color produced in the chlorine determination than the others studied.

Permanent standards for many different determinations are commercially available at the present time. Included are single standards and sets of standards, such as those for *p*H indicator transformations and for many commonly determined industrial and clinical constituents. Examples of series of standards are illustrated in Figs. 3·15 and 3·16.

So-called permanent solutions, whether self-standards or artificial standards, should be watched for possible fading or change of hue. The author has encountered an occasional change of this kind in commercial standard series.

3·12 Solids. In recent years there has been a decided trend toward the use of solid color standards. In general, they are made of transparent glass or of paper; but other materials, such as various ceramic and plastic products, seem to have possibilities.

Because of their generally assumed color stability and ease of handling, glass standards have achieved considerable popularity. There has been some controversy between the producers of such glass standards and those favoring solution standards. The periodical literature may be consulted on the details. A critical analyst will check by other means determinations involving glass standards in order to determine whether they are satisfactory for the purpose employed.

Tintometer, Ltd., and Hellige, Inc., have contributed extensively to the promotion of transparent glass standards in either disc or plate form. New methods of manufacture to meet rigid specifications of optical clarity and color have aided greatly in the preparation of these standard glasses. In the roulette form illustrated in Fig. 3·9 each circular holder contains a standard series combination for a particular constituent.

Extensive work has been carried out at the National Bureau of Standards in calibrating glass color standards and in studying the errors likely to be encountered in their use (*19*, *20*, *24*, *32*, *40*) for certain purposes.

Paper standards may be as simple and crudely quantitative as the familiar litmus test paper. Every chemist learns that the paper turns blue in an aqueous solution whose *p*H is somewhat above 7 and pink when the *p*H is somewhat below 7. Closer estimation of *p*H is possible with nitrazine paper, for example. A simple color chart is furnished with it to show colors, at intervals of about 0.5 *p*H unit, from 4.5 to 7.5, with which one may compare a strip of the paper moistened with the solution to be tested. Such papers are impregnated with the active color reagent.

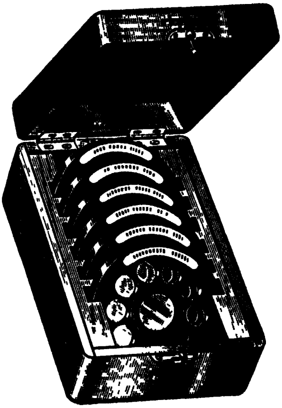


FIG. 3·9. Color comparator discs. (Courtesy of Hellige, Inc.)

Somewhat more refined is the type of color chart, mentioned in Section 3·14, in which a tube of the unknown colored solution is viewed vertically to find a match with one of the series of standards. Since the optical conditions of the solid, reflecting standard are so different from those of the liquid, transmitting unknown, good-quality color measurements cannot be expected. The original, painted standards, made to approximate as closely as possible the colors of a system containing the desired constituent, are reproduced in quantity by three-color printing.

3·13 Methods of comparison. The means employed in making quantitative determinations by comparison of the color of the unknown with that of a standard range all the way from visual observation of two spots on a filter paper to the use of high-class optical instruments.

As might be expected, each procedure has its place. The capable analyst knows what can be done by the various kinds of processes and what his particular problem is. Assuming that he has the various kinds of means available, he would select the one most appropriate to give the information desired.

In succeeding sections there is a brief description of the four principal kinds of comparison techniques.

3·14 Standard series type. One of the earliest types of colorimetric methods, still the most widely used and the least expensive in required apparatus, is that in which the unknown is compared with a series of standards. Usually this series ranges in concentration from somewhat lower than that of the unknown to somewhat higher. A colorimetric match is finally made between the unknown and the standard having as nearly as possible the same concentration. Since solutions so matched are viewed through equal depths or thicknesses. Beer's law need not hold for this type of method. Many devices, ranging from very simple ones to those of some optical complexity, have been developed to simplify and facilitate this comparison. The items of special importance are the standards and the means used for matching. The general characteristics of the standards have been considered in previous sections.

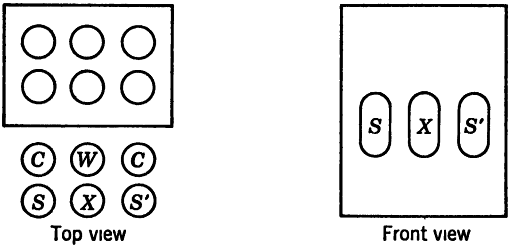


Fig. 3·10. Color comparator block.

Test tubes. Where permanent standards are not desired, the simplest and least expensive containers for liquid standards and unknowns are test tubes. Tubes to be used in this technique should be selected for uniform diameter and wall thickness. Careful observation under standard illumination should give fairly accurate results.

Various kinds of comparator blocks are used to hold the tubes. Such devices have been widely used in *p*H determinations by comparison of the unknown solution with standard buffer solutions. More recently use has been made of these devices in some types of biological analyses, particularly where the original sample is colored.

The type of comparator block suggested by Clark (*10*) is illustrated in Fig. 3·10. The vertical holes should be just large enough to receive the test tubes, in parallel rows and as close together as practicable. In order to view the solutions, horizontal holes usually about 10 mm. wide and 20 mm. high should pass perpendicularly through the vertical holes.

The block, both interiorly and exteriorly, should be painted or stained a dull black.

There are several ways of using the block illustrated. When the sample is colorless, the front row only is used. Tube X represents the unknown, and S and S' are the two standards having a concentration estimated nearest the unknown. The comparison may be accurately made when the block is illuminated from the rear. When the unknown sample is moderately colored or slightly turbid, a comparison may be made in the same manner by superimposing the back row of tubes on the front row. The control tubes C compensate the color or turbidity originally present in the sample; the unknown tube is X, and the tube W behind the unknown contains water to maintain a constant thickness of solution through which the color is viewed. A similar block with three rows of holes has been used for measuring colored indicator samples in the determination of pH by the Gillespie method and is described later.

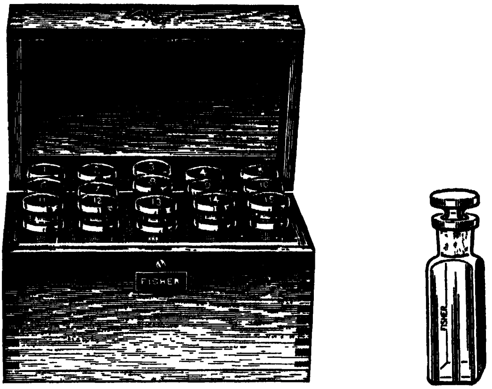


Fig. 3·11. Permanent color standard bottles. (Courtesy of Fisher Scientific Company.)

Comparison bottles. If a cheap but convenient set of permanent standards is desired, the solutions may be sealed in small round or square bottles. Figure 3·11 illustrates a set of square bottles. All self-standard solutions should be prepared under conditions identical with those used in the preparation of the unknown.

The standard bottles should be placed in a row in front of a light source, leaving room between each pair of bottles to interpose the unknown in a similar bottle. The bottles containing the standards can be sealed with wax or paraffin, and the standards can be used until fading begins.

Standard tubes. In 1922 the Committee on Guaranteed Reagents and Standard Apparatus of the American Chemical Society (*11*) recommended certain standard tubes for color-comparison work. These include three forms, as illustrated in Fig. 3·12:

1. Nessler tubes, meeting American Public Health Association standards, in sets of 6 or 12 (*1*).

2. Eggertz tubes without stoppers, with a capacity of 30 ml. or 50 ml. Sets should include either 2 or 4 tubes.

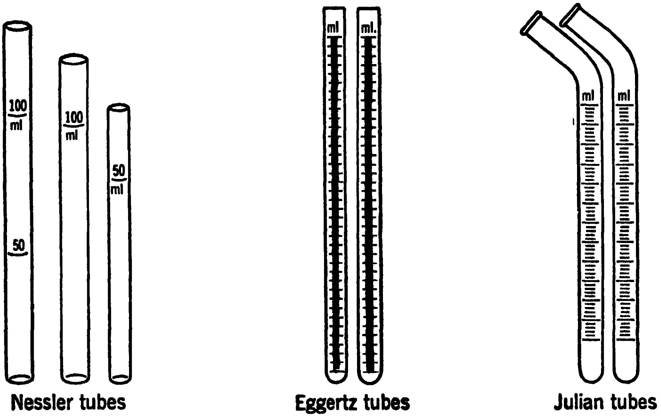


FIG. 3·12. Standard tubes for color comparators.

3. Julian tubes graduated from 5 ml. to 30 ml. or from 10 ml. to 50 ml. in 0.1-ml. steps. These sets also should consist of 2 or 4 tubes.

Both Eggertz and Julian tubes are long tubes of small bore and are carefully graduated in 0.1-ml. steps. The glass is carefully selected and must be of uniform thickness. The graduation marks are carefully etched in the glass. The sets must be matched in bore and glass thickness. Both types are used in the determination of carbon and manganese in steel.

The American Public Health Association has established standards for Nessler tubes as follows: the 50-ml. mark on 50-ml. tubes should be approximately 200 to 250 mm. from the bottom, and the 100-ml. mark on 100-ml. tubes should be approximately 275 to 325 mm. high. The graduation marks on a set of tubes should not vary more than 6 mm. A graduation of 50 ml. is permissible on the 100-ml. tubes. The tubes must be made of clear, flawless glass and have polished flat bottoms, preferably fused on. Clarity of optical field is thus enhanced, since both sides of the bottom are polished.

The above specifications are for "tall-form" Nessler tubes. The "short-form" tube, which varies only in bore, the 50-ml. mark of the 50-ml. tube being placed approximately 120 mm. above the bottom and the 100-ml. mark on the corresponding tube being approximately 150 mm. high, is no longer standard with the American Public Health Association.

Many devices have been invented for viewing Nessler tubes containing colored solutions. As early as 1889, the J. H. Berge Company catalog (*5*) illustrated a simple in-line *Nessler tube rack*. This arrangement has remained comparatively unchanged in design through the years, and one may be found in most analytical laboratories today. A mirror near the bottom reflects light up through the tubes. The unknown is compared with various standards by placing it adjacent to successive standards until a match is found.

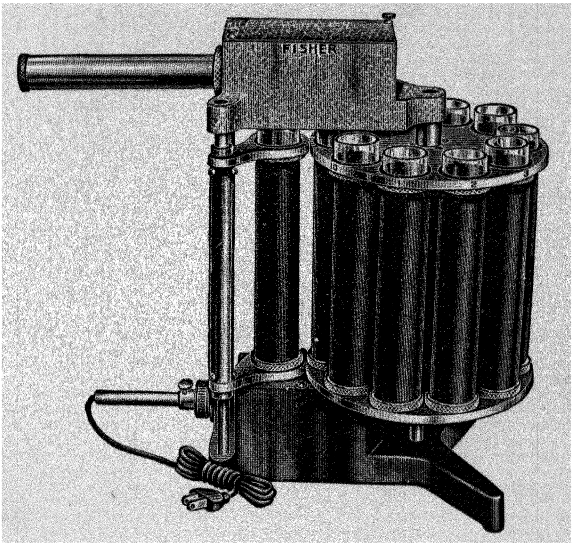


FIG. 3·13. Roulette Nessler tube comparator. (Courtesy of Fisher Scientific Company.)

In a more modern type of comparison device, illustrated in Fig. 3·13, the unknown tube may be compared rapidly with any one of ten standards in similar Nessler tubes by rotating the circular case. The base contains a built-in light source. The standards are accurately placed in the optical system with respect to the unknown by a notched spring device. Extraneous light is excluded by placing the tubes in metal shells mounted in the drum.

The optical system of this instrument is so arranged that light beams enter the bottom of the Nessler tubes, pass through the unknown and one of the standard solutions, then converge, and are observed in juxtaposition in an eyepiece. The comparison is greatly facilitated by bringing both images side by side in a single field of vision. Figure 3·14 shows the effect obtained.

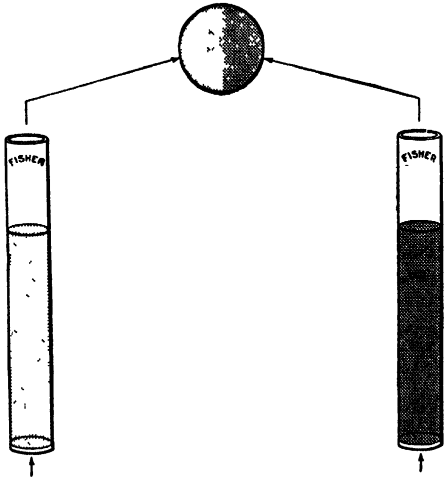


FIG. 3·14. Juxtaposition of light beams in eyepiece of roulette type comparator. (Courtesy of Fisher Scientific Company.)

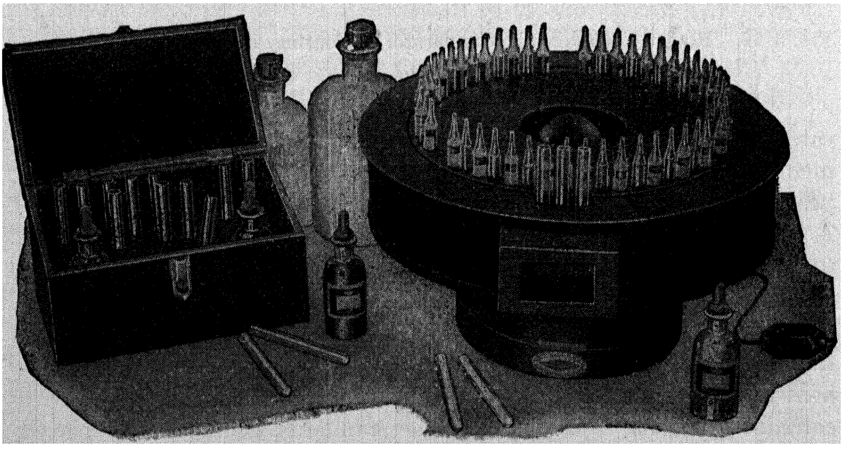


FIG. 3·15. Roulette comparator with permanent liquid standards. (Courtesy of LaMotte Chemical Products Company.)

Instruments have been devised also for the use of *permanent liquid standards* in sealed tubes. Such an instrument, with the required incidental equipment, is shown in Fig. 3·15. Standards for an instrument such as this can be supplied for a great number of determinations, such as *p*H, chlorine, thiocyanate, sulfathiazole, hemoglobin, and phenolsulfonphthalein. Usually the standards and the required reagents for a specific analysis can be purchased as a unit kit. The standards normally can be utilized in an instrument designed for other tests, particularly those for *p*H study.

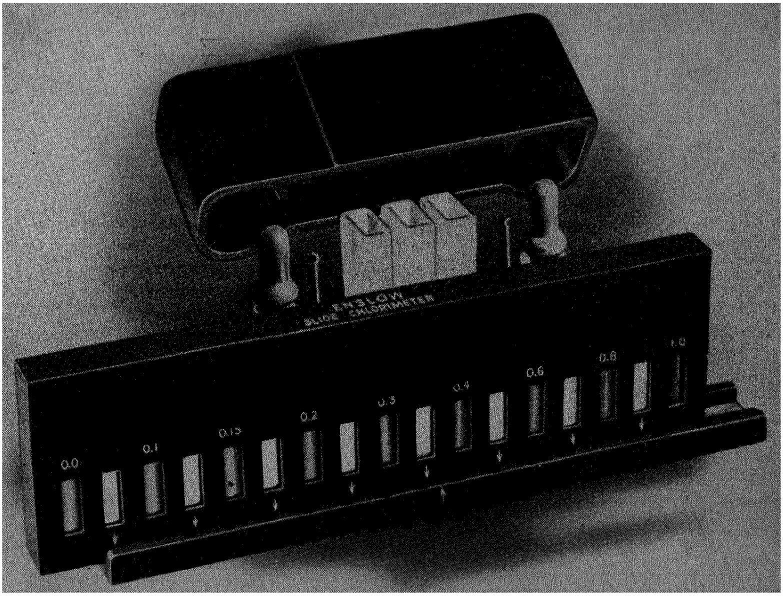


FIG. 3·16. Slide comparator for the determination of chlorine. (Courtesy of W. A. Taylor & Company.)

Slide comparators using permanent liquid standards have been devised for many colorimetric methods. An example of such a comparator, designed for determining residual chlorine in water, sewage, and industrial wastes, is shown in Fig. 3·16. The instrument consists of two principal parts, a base and a slide, both molded from plastic. The base contains three Lucite cells with markings at 11.5 ml., two vials of *o*-tolidine solution, and two 0.5-ml. pipets. The color standards are alternated with vials of distilled water and range from 0.0 through 0.1, 0.15, 0.2, 0.3, 0.4, 0.6, 0.8, and 1.0 p.p.m. of chlorine. The base of the comparator may be used with slides showing different ranges of chlorine or for other tests, such as *p*H.

The discussion of apparatus and methods used in standard series types of comparisons has been limited thus far to liquid standards. It is not intended to imply that the standards employed in such comparisons must be liquid; on the contrary, the use of solid standards is becoming more and more common as new methods are devised for producing such materials capable of meeting rigid specifications (*22*).

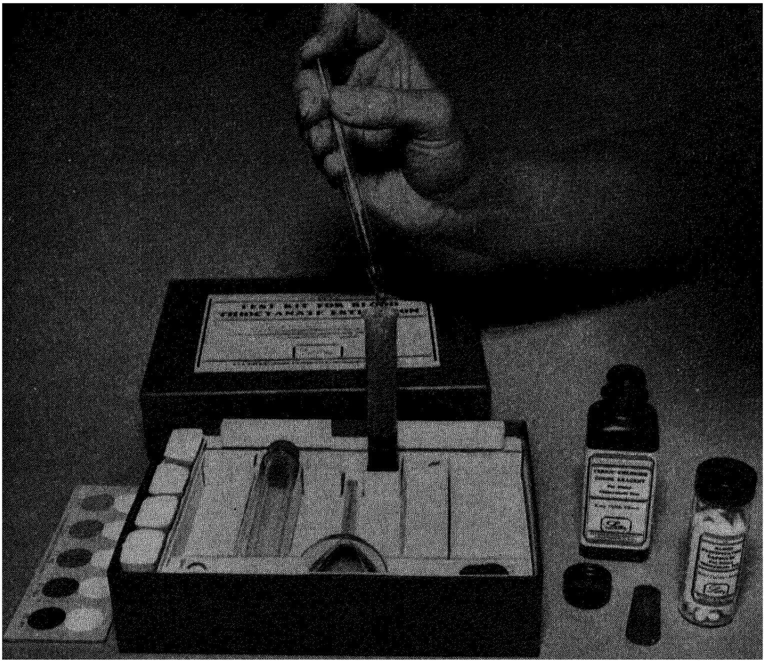


FIG. 3·17. Colorimetric test kit for the determination of thiocyanate in blood. (Courtesy of Eli Lilly & Company.)

One of the simplest standards available in a standard series method is simply a *printed color chart.* Master standards are prepared by the analyst, and the colors are duplicated on paper by a commercial artist. Printing inks may be prepared from these color squares, and large quantities of charts can be printed, using the special inks. It must be remembered that a high degree of absolute accuracy is not always desired in a method. Often it may be sufficient to know only whether a solution is acidic or basic, and a simple test with litmus paper may be just as effective as a much more complicated test using a spectrophotometer.

In the clinical treatment of many illnesses, the use of a drug must be controlled by careful observance of the amount of the drug excreted by the body. Such is the case in the treatment of certain diseases with thiocyanate compounds. Figure 3·17 illustrates a simple self-contained unit for determining thiocyanate in blood. After removal of proteins from a very small amount of blood containing thiocyanate, a filtered aliquot is diluted to a standard volume and a red color is developed by the addition of ferric ions. The color is compared with a standard color chart by looking down through a specified thickness of solution contained in a specially marked test tube. The accuracy obtained is well within the limits required for controlling the dosage of the medication, and the test can be made in 5 minutes.

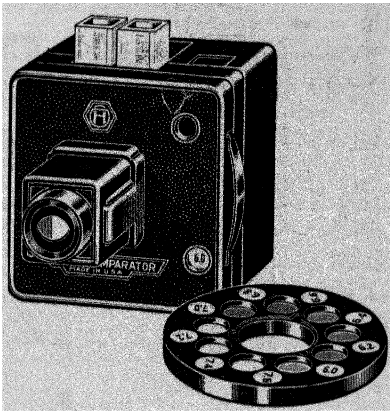


FIG. 3·18. Standard disc comparator. (Courtesy of Hellige, Inc.)

The need for a rapid and convenient method of determining chlorine concentrations in cleaning and sterilizing solutions has led to the development of *test papers* which can be compared with a color chart. These papers are said to be sensitive over the range of 50 to 200 p.p.m. of chlorine, and the color chart may be mounted on the vial containing the strips of paper.

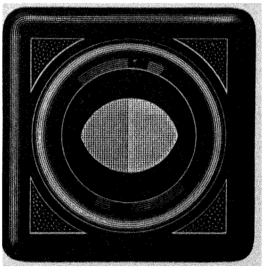


FIG. 3·19. Color fields as seen in disc comparator. (Courtesy of Hellige, Inc.)

More complex devices have been designed for the comparison of a colored unknown solution with glass standards. Figure 3·18 illustrates a general type of such a comparison instrument. In most of these instruments the glass standards are fitted into a *roulette disc* which may be viewed, one standard glass at a time, in juxtaposition with the unknown colored solution. Usually a receptacle is provided in front of the roulette disc in order that lightly colored or slightly turbid solutions may be compensated by inserting a comparator tube of the unknown directly in front of the standard glass. On some instruments provision has been made for attaching a prism device to the comparator in order to bring the color fields adjacent, as shown in Fig. 3·19. Roulette standard color discs are usually interchangeable in the comparator. One manufacturer lists color disc standards for twenty-four different *p*H indicators, seventeen standards for water analysis, and many more for clinical and other industrial methods, all capable of being used in the same comparator.

Various modifications of such devices permit their use in micro work. Depths of solution through which the color may be viewed range from as low as 6 mm., requiring only 0.25 ml. of solution, to more than 40 mm. for macro work. Both pocket and laboratory models are available. Recently, at least one manufacturer has added a standard light source in the form of a special light and filter which can be clamped on the comparator.

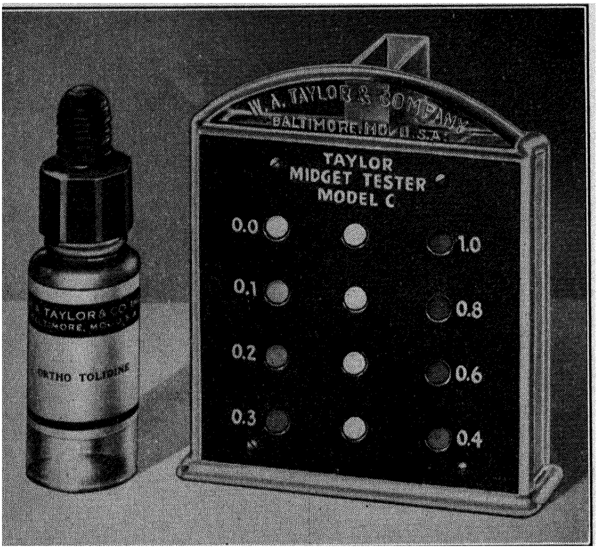


FIG. 3·20. Comparator using fixed glass standards. (Courtesy of W. A. Taylor & Company.)

A simplified instrument of this general type, wherein the glass standards are fixed and located on each side of the tube containing the unknown is shown in Fig. 3·20.

***p*H determination.** One of the most important colorimetric methods utilizing standard series procedures is the determination of *p*H. The method depends on the ability of certain chemical compounds known as "indicators" to change in hue with a change in the concentration (activity) of the hydrogen ions in solution. For example, bromocresol green appears yellow at *p*H 4.0; as the concentration of hydrogen ions decreases and the pH increases, the hue of the solution changes through green to blue at pH 5.6. It should be noted that the entire color change of this indicator occurs on the acidic side of neutrality. Some indicators, such as bromothymol blue, show a characteristic color change at or very near the neutral point, pH 7.0.

Since these changes in hue with change of pH in solutions containing known amounts of indicator are reproducible, it is possible to determine the pH of any aqueous solution colorimetrically. One needs only the indicators required to cover the possible pH range and a means of comparison of the unknown and the standards.

A simple explanation of this change of hue of a solution with change of hydrogen-ion concentration may be made if the indicator is considered a weak acid or weak base. Assuming the indicator to be a weak acid, HIn, we may immediately assume that a reversible reaction is occurring in the solution

$$\underset{\text{Unionized}}{\mathrm{HIn}} \rightleftarrows \underset{\text{Iogenic form}}{\mathrm{HIn'}} \rightleftarrows \underset{\text{Ions}}{\mathrm{H^+ + In^-}}$$

wherein the ion In^- has one color and the unionized form HIn has another color or none at all.

Considering the constants involved, we find

$$\frac{[\mathrm{HIn'}]}{[\mathrm{HIn}]} = k_1$$

$$\frac{[\mathrm{H^+}][\mathrm{In^-}]}{[\mathrm{HIn'}]} = k_2$$

Multiplying k_1 by k_2,

$$\frac{[\mathrm{H^+}][\mathrm{In^-}]}{[\mathrm{HIn}]} = k_1k_2 = k_{\mathrm{In}}$$

Then

$$[\mathrm{H^+}] \cdot \frac{[\mathrm{In^-}]}{[\mathrm{HIn}]} = k_{\mathrm{In}}$$

Now it may be seen that the ratio $[In^-]/[HIn]$ will vary as the concentration of hydrogen ions changes, since the product of the two equals a constant. A change in color is evident upon the change of the above ratio; hence, since a change in concentration in the ratio is dependent upon a change in the concentration of the hydrogen ions, $[H^+]$, the color change may be shown to be directly dependent upon the change of pH.

A simple means of using this method consists of adding a specific volume of the selected indicator to a series of tubes containing equal volumes of buffer solutions—solutions containing known concentrations of hydrogen ions. The same volume of indicator is added to a tube containing the unknown, the total volume being identical with that of the buffer solution. By the use of a comparator device, such as the comparator block described previously, the unknown may be compared with the standards and a match secured. When the unknown is darker than one standard tube and lighter than the next succeeding tube, the average of the two standards will be the *p*H of the unknown. For accurate *p*H determinations Clark and Lubs (*10*) proposed that the buffer solutions should be not more than 0.2 *p*H unit apart. Full directions for the preparation of such series are given in various publications (*6*, *10*, *26*).

Although a large number of indicators have been proposed for such colorimetric *p*H determinations, the selected group listed in Table 3·1 is most commonly used.

TABLE 3·1 SELECTED INDICATORS

Common Name	*p*H *Range*	*Color Change*	*pK*	*Concentration, per cent*
Thymol blue	1.2– 2.8	Red-yellow	1.7	0.008
Bromophenol blue	3.0– 4.6	Yellow-lavender	4.1	0.008
Bromocresol green	4.0– 5.6	Yellow-blue	4.7	0.008
Bromocresol purple	5.2– 6.8	Yellow-purple	6.3	0.012
Bromothymol blue	6.0– 7.6	Yellow-blue	7.1	0.008
Phenol red	6.8– 8.4	Yellow-red	7.7	0.004
Cresol red	7.2– 8.8	Yellow-red	8.1	0.008
Thymol blue	8.0– 9.6	Yellow-blue	8.8	0.008
Thymolphthalein	9.3–10.5	Colorless-blue	9.2	0.008

Another simple method of *p*H determination, proposed by Gillespie (*21*), eliminates the necessity of the buffer solutions discussed above and may be seen to be a milliliter-ratio modification of the former. An approximate *p*H value of the unknown is determined by use of a simple wide-range indicator, to show the proper indicator to select. Comparison tubes are arranged in a two-row comparator block, one row containing a suitable acid and the other a suitable base to transform the indicator to the acidic or basic form, respectively. Indicator is added to the tubes in ratios such that the total amount of indicator in the acidic tube and in its complementary basic tube is constant for all tube combinations. Thus, if the acidic tube contained 0.10 ml. of a specified indicator, the basic might contain 0.90 ml.; if the acidic contained 0.70 ml., the basic would contain 0.30 ml. The unknown tube, containing the full amount of indicator normally divided between the acidic and basic tubes, is viewed together with a water "blank" tube in order to maintain a constant thickness of solution. The unknown is matched with the combined acid-base standards in a two-row comparator block, and the *p*H value is calculated thus:

$$pH = pK + \log\left(\frac{\text{ml. in basic form}}{\text{ml. in acidic form}}\right)$$

The *pK* values for the common indicators are given in Table 3·2. A constant must be added to or subtracted from the *pK* value, depending upon the milliliter-ratio in the matching tube pair. The table contains the constants for all of the milliliter-ratio relationships.

Table 3·2 Indicator Milliliter-ratio Relationships

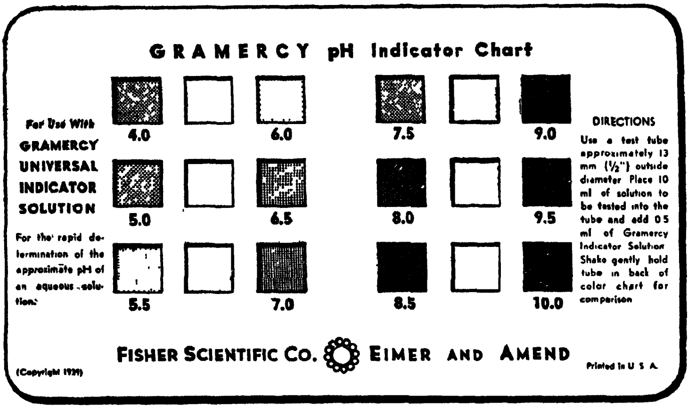
Milliliter-ratio	*Per Cent Neutralized*	*Increment to pK Value*
0.10/0.90	9.1	−1.0
0.15/0.85	13.7	0.8
0.20/0.80	20.0	0.6
0.30/0.70	28.5	0.4
0.40/0.60	38.7	0.2
0.50/0.50	50.0	0.0
0.60/0.40	61.3	+0.2
0.70/0.30	71.6	0.4
0.80/0.20	80.0	0.6
0.85/0.15	86.3	0.8
0.90/0.10	90.9	1.0

A modification by the Bausch and Lomb Optical Company of the Duboscq balancing comparator (see Section 3·17) provides for a more elegant application of the essentiality of the Gillespie milliliter-ratio method.

It cannot be overemphasized that many analytical determinations require an adjustment of *p*H which does not require the high degree of accuracy described above. Very often common *p*H test papers will give the information desired with a minimum of effort. Such test papers are available in 1½-in. strips or in rolls. Dispensers may be secured for certain *p*H test papers with color charts indicating the *p*H values of various colors which may appear mounted on the dispenser.

Universal indicator solutions may be secured for determining approximate *p*H values of solutions. Figure 3·21 illustrates a universal indicator and the accompanying color chart. With such an indicator a specified amount of the indicator is added to approximately 10 ml. of the unknown solution in a test tube, and the color produced is compared with the color chart. The pH of the matching color is indicated on the chart.

Many modifications of instruments and devices for comparison of an unknown with a standard series have been developed during recent years by analysts engaged in pH determinations. Special flasks having sidearms for insertion into comparator blocks for following pH during titrations, special comparator blocks permitting a portion of the titrated solution to be compared with pH standards, even complete laboratory units containing a mounted comparator, source of illumination, and all necessary equipment and reagents for accurate pH determinations—all such modifications and improvements are indicative of the continued importance attached to standard series comparison methods. Examples are illustrated in Fig. 3·22 to 3·24.



GRAMERCY pH Indicator Chart

For Use With
GRAMERCY
UNIVERSAL
INDICATOR
SOLUTION

For the rapid determination of the approximate pH of an aqueous solution.

4.0		6.0	7.5		9.0
5.0		6.5	8.0		9.5
5.5		7.0	8.5		10.0

DIRECTIONS

Use a test tube approximately 13 mm (½") outside diameter Place 10 ml of solution to be tested into the tube and add 0 5 ml of Gramercy Indicator Solution Shake gently hold tube in back of color chart for comparison

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FIG. 3·21. Universal indicator and color chart. (Courtesy of Fisher Scientific Company.)

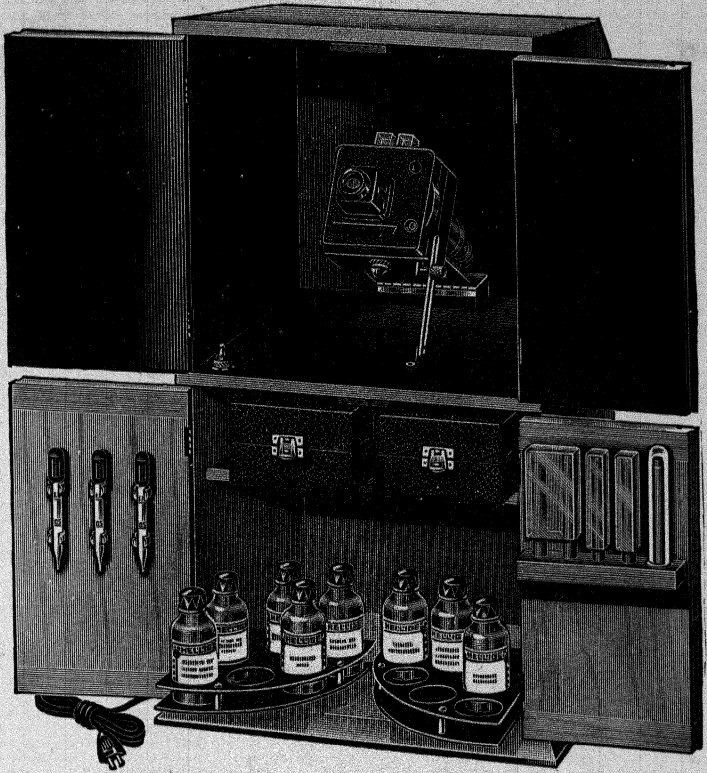

FIG. 3·22. *p*H laboratory. (Courtesy of Hellige, Inc.)

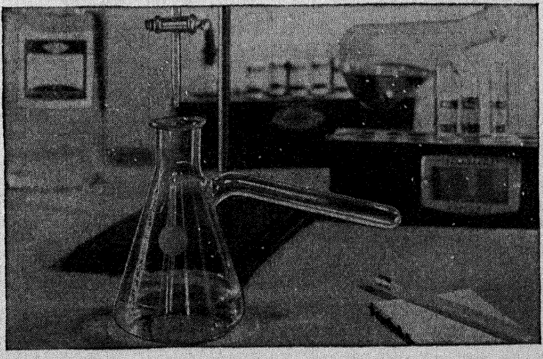

FIG. 3·23. Special flask for *p*H end-point titrations. (Courtesy of LaMotte Chemical Products Company.)

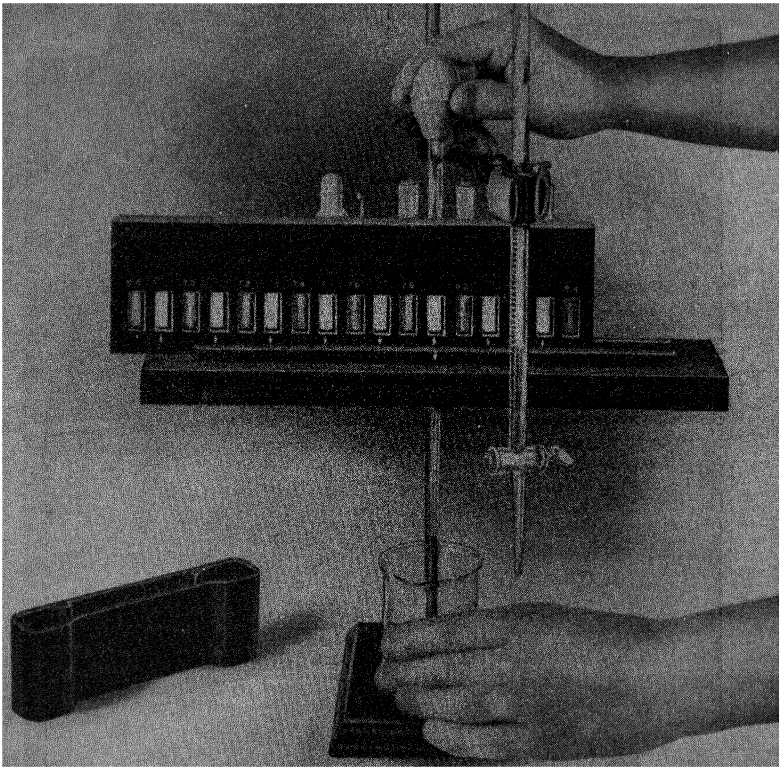

FIG. 3·24. *p*H slide titrator. (Courtesy of W. A. Taylor & Company.)

3·15 Duplication type. As defined earlier in this chapter, the duplication type of method depends upon reproducing or "duplicating" the color of the unknown by adding known amounts of the desired constituent to another system containing the same reagents and volume as the unknown until a colorimetric match is obtained.

Since both the known and the unknown colored systems are identical at the final match in both concentration of desired constituent and depth of solution viewed, it may be seen that Beer's law need not hold for these methods.

Matched Nessler tubes meeting the same physical standards as set forth under standard series apparatus normally are used in duplication methods. The unknown colored solution is first developed and roughly estimated by inspection. Identical amounts of reagents are placed in the second Nessler tube, and the volume is brought up almost to that of the unknown tube. Known amounts of the desired constituent are added from a buret or by weight until, after thorough mixing, the colors match. The known tube is then diluted to the exact volume, and the match is again checked and adjusted if necessary. It then follows logically that a measure of the desired constituent added to the known tube gives directly a measure of the amount in the unknown. This method actually consists of titration to a specified colorimetric end point. The desirability of a permanent standard solution of the desired constituent is obvious.

3·16 Dilution type. The dilution type of method depends upon securing a colorimetric match of the known and unknown solutions by adding solvent to the more concentrated system until the color intensities are equal.

Care should be taken to insure that the tubes used for such comparisons are matched in thickness of glass and internal diameter. The two tubes are compared through the same thickness of solution by viewing horizontally rather than vertically. Matched Nessler tubes provide a very simple type of such comparison tubes.

As in other methods, special devices have been designed to simplify the final comparison. The Myers colorimeter is illustrated in Fig. 3·25. Here the comparison is made through a small opening against a white glass background.

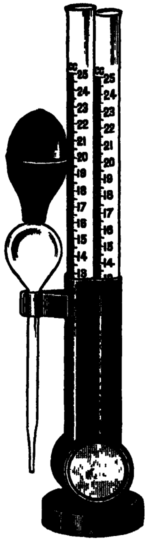

FIG. 3·25. Myers colorimeter. (Courtesy of A. H. Thomas Company.)

In calculating the results of the method it may be seen that, since the concentration of the desired constituent is fixed in the known, the concentration in the unknown is inversely proportional to the ratio of the volumes when a colorimetric match is secured. Here, obviously, Beer's law must apply, or correction be made for divergence from it.

3·17 Balancing type. The most elaborate type of method with respect to the apparatus used, and yet one of the simplest with respect to the use of the equipment, is the balancing type. This method depends upon comparing the intensity of color in an unknown solution with that of a single known solution, the depth through which one solution is viewed remaining constant and the depth through which the other is viewed being varied until a colorimetric match is secured.

Since the concentrations of the known and unknown may be dissimilar and the depths of solution through which the colors are compared may vary widely, Beer's law must hold for the colored system to permit reasonable accuracy, or compensating corrections must be established. If the system conforms to Beer's law, the calculations involved are very simple, since the concentrations of the desired constituent in the known and unknown are inversely proportional to the depths of the solutions through which the intensities of color are viewed at the point of colorimetric match.

The simplest type of balancing apparatus consists of two matched Nessler tubes, or other similarly graduated cylinders, and a white paper or paint background. One tube may be filled to a certain depth with the known solution and placed above the background. The unknown solution is slowly poured into the second tube until the point of colorimetric match is reached, when the tubes are viewed vertically. If the tubes have the same internal dimensions, then the concentrations are inversely proportional to the volumes of the solutions. If the diameters and markings of the tubes are not identical, then the concentrations are inversely proportional to the depths of the solutions through which the color is viewed. For highest accuracy the concentrations of the constituent in both known and unknown should be similar.

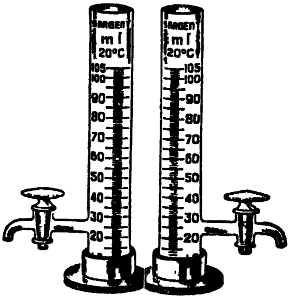

FIG. 3·26. Hehner cylinders.

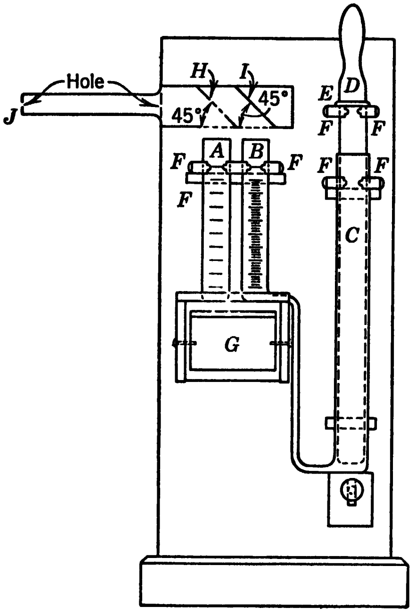

FIG. 3·27. Schematic diagram of Kennicott-Campbell-Hurley comparator.

Nessler tubes modified to facilitate balancing are known as Hehner cylinders. As shown in Fig. 3·26, they have stopcocks near the base. A known solution is placed in one cylinder, and the unknown in the other cylinder. After placing the cylinders over a source of illumination, the solutions are viewed vertically. To secure a colorimetric match it is necessary only to open the stopcock of the cylinder containing the solution of greater intensity of color and draw off this solution until the match is made. As before, the concentrations of the solutions vary inversely with the depth through which the colors are viewed. Specifications for this type of cylinder are similar to those for Nessler tubes. The glass used must be free from defect; the markings must be clear and sharp and reasonably close together on matched cylinders. Internal diameters must meet specified requirements.

In the next level of improvement in balancing type devices is found an instrument wherein the length of one column of solution is fixed, whereas that of the other is varied by mechanically adding or removing the liquid from the viewing tube (*8*). A cross section of such an instrument is illustrated in Fig. 3·27. The left tube is designed for a fixed volume of the unknown and hence is marked only at 5-mm. intervals. The standard solution is placed in the right tube, *B*, permanently connected to the reservoir *C* by a glass tube. Tube *B*, in which the level of the standard is raised or lowered by moving the plunger *D* up or down in the reservoir, is marked off in 1-mm. intervals. Since the tube *B* and the reservoir *C* are connected only by glass, and the plunger is also glass, the liquid used as a standard comes in contact only with glass, thus preventing any contamination due to contact with the container. The plunger is provided with a rubber collar *E* placed in such a position that the plunger cannot accidentally strike and break the bottom of the reservoir. The comparison tubes and reservoir are held in place by spring clips *F* to allow removal for cleaning. The light for illumination is reflected up through tubes *A* and *B* from the adjustable mirror *G*. The light passing up through the tubes is reflected by the two mirrors *H* and *I*, which are cemented on brass plates sliding in grooves cut at an angle of 45° in the sides of the optical box. The mirror *H* is so mounted and cut that it reflects one-half of the circular field of light coming through tube *A*. The light passing through tube *B* is reflected horizontally by mirror *I*, but half of this light is cut off by the mounting of mirror *H*.

The image of half of tube *B* is then seen in juxtaposition to the opposite half of the image of tube *A* and is observed through a tube and eyepiece *J* containing diaphragms proportioned so that only the images of the bottoms of the tubes are seen. Thus no interference will occur from light reflected from the sides of the comparison tubes.

One using this comparator views a circular field divided by an almost imperceptible line when the solutions are matched. Raising or lowering the plunger causes the right half of the field to become lighter or darker.

The concentration of the unknown in tube A varies inversely with the depth of the standard solution in tube B, the depth being read on the millimeter scale of tube B.

The Duboscq comparator. Probably the best-known type of balancing instrument is the Duboscq comparator. Figure 3·28 illustrates the general design of such an instrument. A represents a mirror reflecting light from the source of illumination up through the glass-bottom comparison tubes B and B'. C and C' are glass prisms around which the comparison tubes can be raised or lowered by the racks and pinions D and D' in order to vary the viewing depth of the colored solutions. E and E' are prisms for bringing the beams of light together in a divided circular field in the eyepiece F. The side view shows the vernier scale located on the rack and pinion device on which the viewing depth is read.

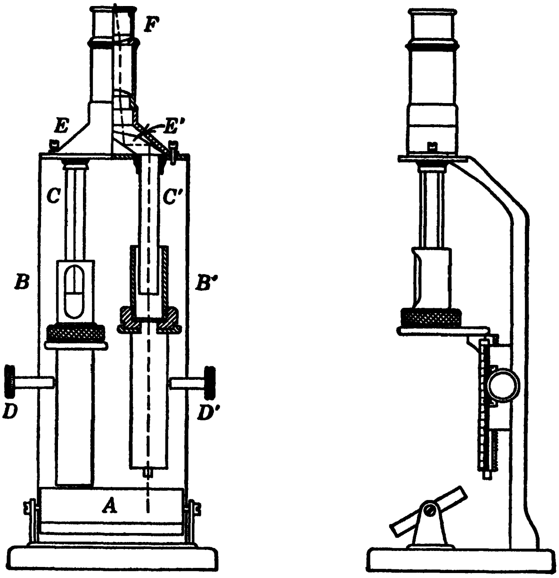

FIG. 3·28. Schematic diagram of Duboscq comparator.

The principle involved in the Duboscq, or other depth-balancing, instrument may be considered in terms of equation (2·14). For the two cells, 1 and 2, containing two solutions of different concentrations, we may write

$$\log \frac{I_0}{I_1} = a_s b_1 c_1$$

$$\log \frac{I_0}{I_2} = a_s b_2 c_2$$

I_1 may be made equal to I_2 by adjustment of the thickness of the solutions. Then, if one divides the two equations, there results the expression

$$b_1c_1 = b_2c_2$$

If c_1 is known and c_2 is desired, from the measured thicknesses, b_1 and b_2,

$$c_2 = \frac{b_1c_1}{b_2}$$

As discussed under Section 3·7, mechanical or equipment errors, the comparator should be treated as a fine instrument. It should be kept clean, and the prisms and tubes in contact with solutions being compared should always be cleaned and wiped dry. In using the instrument it should always be checked before making determinations. Either daylight or artificial illuminators may be used as sources of illumination. If daylight is used, the mirror should be adjusted to obtain a satisfactory indirect light.

Many modifications have been made on the physical appearance of the original Duboscq comparator, but throughout all these changes the basic principles have remained the same. The two-stage compensation Leitz instrument provides for samples with extraneous color (*39*). The Leitz "Universal" instrument is a three-stage assembly.

One of the recent modifications is an inclined eyepiece to permit the operator to work in a more comfortable position. Figure 3·29 illustrates a modern model of the comparator which includes the inclined eyepiece, illuminated scales reading to 0.1 mm., and a built-in light source in the base of the instrument.

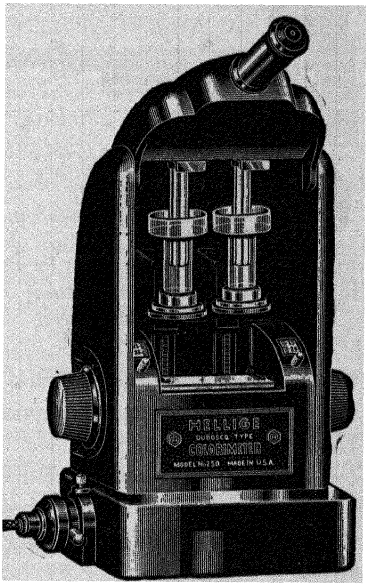

FIG. 3·29. A modern Duboscq type comparator. (Courtesy of Hellige, Inc.)

Other modifications include various types of illuminated vernier scales read from the eyepiece position, armored tubes to prevent breakage, special cups for dilute solutions, and an adjustment by which the instrument can be converted from 50-mm. to 100-mm. tubes.

As micromethods of analysis developed, the need arose for microtypes of comparators. Among the interesting types developed for specialized work, one has been designed for microwork on blood chemistry. The specially graduated 1-ml. glass syringe is attached to the tube for the unknown solution. Figure 3·30 illustrates this instrument. In it provision is made for the use of glass standards.

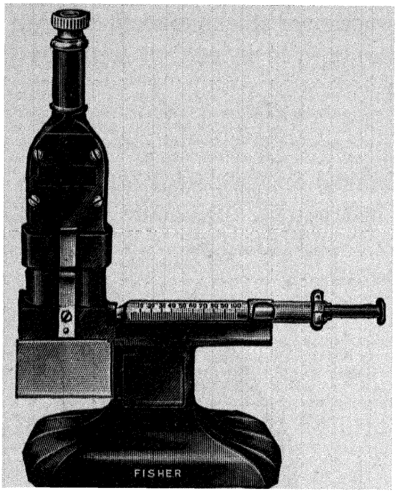

FIG. 3·30. Microcomparator for use in blood chemistry. (Courtesy of Fisher Scientific Company.)

The use of permanent glass standards is now feasible in many balancing instruments. One manufacturer provides such standards for use with a Duboscq comparator in the determination of hemoglobin, icterus index, bilirubin, and glucose in blood, and for other uses in clinics and hospital laboratories. These standards may be used in conjunction with the tubes of the comparator or, in some cases, may be placed in the optical system of the comparator in conjunction with the ocular prisms. In either method, the glass standard acts as the standard tube and is considered the color of a standard liquid with the depth of solution set at a specified figure. As discussed under sources of error, such standards require specific types of illumination to retain their standard value in the comparison.

Thiel (*39*) modified the Duboscq instrument in order to work in terms of absorbancy indexes by comparison of the unknown solution with a gray standard solution having an index of 0.5.

C·MERITS OF COMPARIMETRIC METHODS

Visual comparator methods of analysis have both advantages and disadvantages in comparison with other types of methods.

3·18 Advantages. As advantages, the following items may be noted:

1. Usually the methods are rapid. Often a determination of a desired constituent may be finished completely by colorimetric methods during the time required for separation in a gravimetric method.

2. They require a minimum of preparation of sample. Usually the only operations required are dissolution and color development. The

problem of separation of the desired constituent, often laborious and time consuming, usually may be eliminated, especially if diverse ions do not interfere.

3. Usually they require only a small amount of sample. In many methods a few milligrams of the desired constituent are sufficient to produce a visually comparable color. If the desired constituent is present in large quantities, simple dilution permits the use of comparator methods.

4. They require a minimum of inexpensive equipment. In the simplest types, test tubes suffice. Even the best comparator instruments are relatively inexpensive, compared with the cost of spectrophotometers or most filter photometers.

5. Highly trained technicians are not required. With some supervision, a nontechnical person may become quite adept at using comparator devices and in matching color intensities.

3·19 Disadvantages. Although comparator methods usually have some of the above advantages over other methods of analysis, they are also susceptible to the following disadvantages:

1. Unless permanent standards are available, the technician must make up new standard solutions as often as daily.

2. The presence of interfering ions often requires extensive preparation of the sample before color development. Certain interfering ions may prohibit the use of existing comparator methods for the determination of some desired constituents.

3. Suitable sources of illumination, particularly for dissimilar permanent standards, may not be available.

4. The sensitivity of comparator methods sometimes is not high enough for the required determination. An absolute accuracy of ±5 per cent may be expected routinely, and even ±2 per cent accuracy may be achieved; but in comparison with good gravimetric methods, for example, this figure may be too high.

The chemist must weigh these advantages and disadvantages against those of other methods, and, knowing the objectives required and the limitations imposed upon the problem, he must select the method most suitable for his requirements.

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4· FILTER PHOTOMETERS

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4·1 Introduction. The discussion in Chapter 5 on spectrophotometry shows that the most complete information about the optical characteristics of a colored substance is obtained by a precise measurement of the transmittancy or reflectance of the substance at every wavelength throughout the visible spectrum. It is because so much useful information may be derived from an abridgment of this technique that filter photometry is so important. Many colored systems of interest and importance to the chemist possess broad and relatively nonselective absorption bands, and consequently their properties may be investigated by light covering a range of wavelengths which can be isolated quite readily with filters. The measurement of transmittancy can be made visually or photoelectrically.

It may be said with a fair degree of certainty that the techniques of the future will be concerned almost entirely with photoelectric methods. In few cases can it be said at this time that photoelectric instruments have been accorded the same degree of careful design and refinement which we find in the best of the visual instruments. The fact remains that there seems to be little room for the further improvement of visual instruments because they have been refined to the point where their convenience and precision are limited by the ability of the eye to distinguish significant differences in intensity. Conversely, photoelectric methods impose practically no fundamental limitations on either precision or speed. The practical limitations really represent a compromise between costly elaboration of design and the immediate demands of the photometric problem.

4·2 Components of photometers. A filter photometer involves six principal factors: (1) a suitable light source; (2) a filter or combination of filters for isolating a narrow band of wavelengths; (3) an optical system for producing a parallel beam of filtered light and, in this assembly, may be included the means for containing the sample and reference substance with means for their alternate exposure to the beam; (4) some means for establishing a photometric balance; (5) a means for indicating the state of balance; and (6) some provision for regulation which, in various cases, may involve stabilization of the light source or compensation for systematic or transient disturbances in the indicating system. The relative importance of these factors is largely a matter of design, and this will be made somewhat clearer in the discussion of specific cases which follows.

It may be well to present at this point some additional reasons why filter photometers can be as useful as the more elegant spectrophotometer. For most of the colored solutions of interest to the analyst in "colorimetric analysis" the absorption bands are quite broad, and therefore there is correspondingly less need for a monochromatic source of light. It is not surprising, therefore, that many of these systems obey Beer's law over a relatively wide range of concentration, even when examined by the light transmitted by a relatively nonselective filter. It is true that a certain degree of empiricism is unavoidable in this technique because, unless the characteristics of the filter and the light source are carefully specified, the transmittancy values cannot be expressed in unambiguous terms, as they can when a spectrophotometer is used. Nevertheless, it is all too evident that an unnecessary degree of empiricism in photometers has been accepted by chemists, and much of this can be eliminated by improved design, especially in the photoelectric instruments. In the latter, it is very important to know precisely how much of the empirical relationship between the instrumental indication and concentration is attributable to instrumental factors and how much is to be ascribed to the chemical vagaries of the system.

Filter photometers, especially of the photoelectric type, offer almost unlimited means for the rapid examination of colored systems with the inherent possibility of automatic recording, control, and the remote transmission of the data. It is evident that the inclusion of the latter refinements also implies mechanical requirements for handling the sample.

4·3 Classification of photometers. Although possibly other bases of classification may have merit, current usage leans heavily to classification according to the kind of receptor used for the transmitted radiant energy. This viewpoint leads naturally to two main subdivisions—visual and nonvisual, or objective, instruments.

4·4 Visual photometers. We may dispose of the visual photometers by citing a few examples of the best practice in this class of instruments. They consist of a light source with means for producing two uniform beams which can be rendered parallel and pass through two absorption cells, after which the beams are combined with a biprism and eyepiece to present a twin photometric field to the observer. A series of highly selective filters, transmitting narrow regions of the spectrum, is interposed between the biprism and the eyepiece, and these may be selected by the observer so that he may view the photometric field at more or less equal intervals throughout the visible spectrum. Some provision is then made for the precise adjustment of the intensity of light passing through the reference cell. In general, one of three methods is used for this purpose: adjustment of the beam by a calibrated diaphragm, by polarization equipment, or by the introduction of a neutral wedge. With this assembly of components, the observer can determine the transmittancy of the system at uniform intervals throughout the spectrum and thus obtain an abridged spectrophotometric curve for the substance. A curve of this kind has no particular value in the spectrophotometric sense; its value resides in the ability of the observer to find the filter which will yield the greatest absorptivity for that system. With this information at hand he is in a position to determine to what extent the system will follow Beer's law.

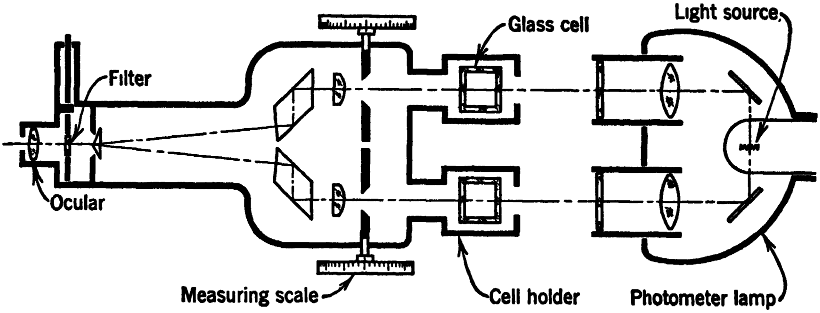

FIG. 4·1. Schematic sectional diagram of Zeiss Pulfrich photometer. (Courtesy of C. Zeiss, Inc.)

Excellent examples of this class of instrument are the Zeiss gradation photometer, a schematic representation of which is shown in Fig. 4·1, the Leitz photometer of Fig. 4·2, and the Aminco photometer of Fig. 4·3. The Zeiss and Leitz photometers are general-purpose instruments, and numerous attachments are available for the measurement of other optical properties including reflectance, gloss, and fluorescence. Their construction is also such that a wide range of absorption cells can be accommodated. The Aminco instrument, which employs a neutral wedge principle, has been furnished in its photoelectric equivalent. The manufacturers of these instruments have supplemented the incandescent light source with a mercury arc, together with a suitable series

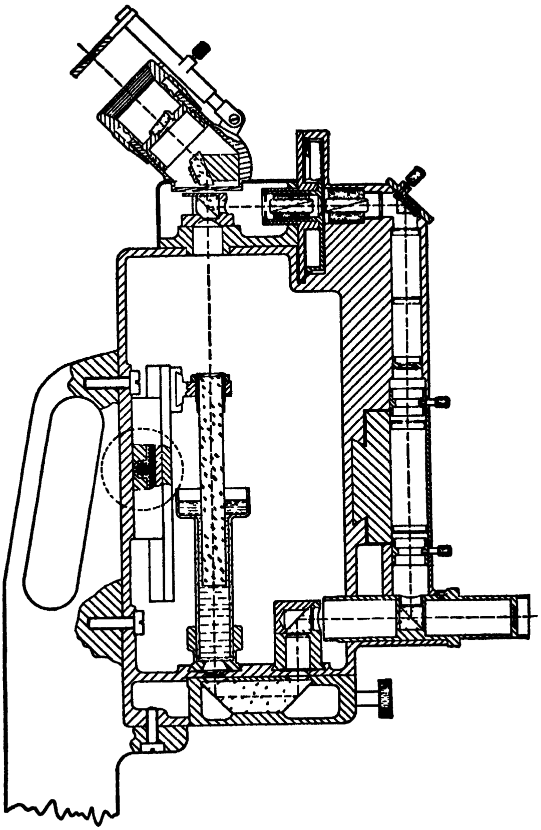

FIG. 4·2. Schematic sectional diagram of Leitz polarization type photometer. (Courtesy of E. Leitz, Inc.)

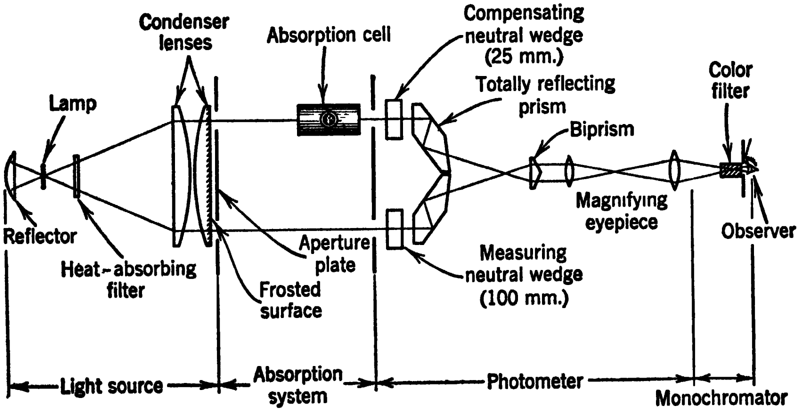

FIG. 4·3. Wedge type photometer. (Courtesy of American Instrument Company.)

of filters for isolating the principal lines of the mercury spectrum. With this choice of illuminant and accompanying filters, one can achieve the rigor and precision of true spectrophotometry but, obviously, within the limitation of the available mercury lines.

4·5 Nonvisual or objective photometers. It cannot be denied that the visual filter photometer is a very useful instrument with a high degree of versatility and flexibility. However, there is an inherent limitation in the ability of the eye to detect intensity differences in a photometric field; but when these differences are detected photoelectrically, such limitations become evident only at several orders of magnitude lower than the visual limit. There are wide variations in the ability of different observers to match a photometric field, and all observers are subject to fatigue. In this respect, photoelectric instruments are not inherently affected by these factors. The photoelectric approach to this problem necessarily differs from the visual method. The earlier attempts to substitute a phototube for the eye relied upon the optics of the visual instrument and used the phototube as a criterion of photometric balance. In general, it is poor practice to attempt to utilize the optics of a visual instrument for a photoelectric method, although some examples of this type are still to be found among commercial instruments.

It is equally unreliable to place too much dependence upon the relationship between light intensity and photocurrent. The ideal photoelectric photometer would put no dependence at all upon the validity of this relationship but would restrict itself to the use of a phototube as a reliable short-term indicator of photometric balance. Under these ideal conditions, the two photometric beams would be alternately and rapidly viewed by a phototube and so adjusted in their respective intensities until the phototube and its associated equipment fail to perceive any difference. For several reasons, mostly economic, the majority of photoelectric photometers do not employ this principle. When a phototube is depended upon for long-term stability in the comparison of the two photometric beams, other problems arise, such as the stability of the light source and of the associated measuring equipment. We find numerous examples among currently available instruments in which the light source is operated from constant-current transformers, and in which other refinements are required to assure stability at least over the time required to compare the two beams. An alternative approach uses two phototubes with appropriate electrical circuits to intercompare the respective photocurrents and thereby minimize the effect of light-source fluctuations. The various circuits which have been proposed achieve this compensation to varying degrees. They will be discussed in detail later.

4·6 Photoelectric receptors. The majority of photoelectric photometers use one of two general types of light-sensitive devices, the barrier-layer cell and the high vacuum phototube. Special forms of phototubes such as the photomultiplier, iconoscope, and Orthicon have found limited application in photometry but will, undoubtedly, exercise considerable influence on photometer design in the future. The prin-

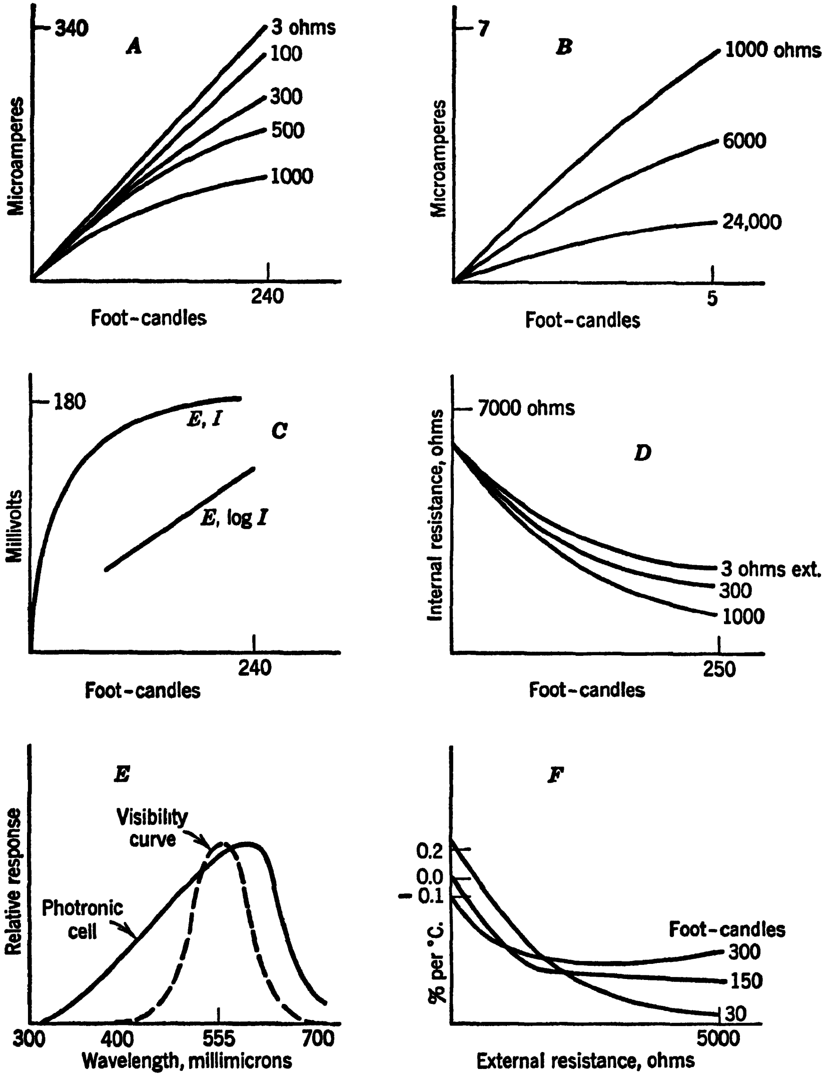

FIG. 4·4. Photronic cell characteristics.

cipal characteristics of these devices will be discussed in order that their application to photometers may be appraised.

4·7 Barrier-layer cells. These cells consist essentially of a plate of copper or iron upon which a semiconducting layer of cuprous oxide or selenium is grown. The semiconductor is covered by a light-transmitting layer of metal—gold, platinum, copper, or lead—which serves as a collector electrode. Upon illumination through the transparent electrode an electron current flows. In this type no auxiliary source of e.m.f. is required. For this reason Lange (*9*) has termed these cells photoelements in analogy with galvanic elements. The more important characteristics are represented in Fig. 4·4.

The photocurrent is very nearly directly proportional to the light intensity for low values of the external circuit resistance, *R* (Fig. 4·4 *A* and *B*). The open circuit e.m.f. intensity relationship is shown in Fig. 4·4 *C*. The linear relationship between *E* and log *I* has interesting possibilities for colorimetry (*16*) and has been neglected or overlooked in American practice, although Lange (*9*) has accounted for it on theoretical grounds and shown that a formal analogy with the Nernst equation for a concentration cell predicts the observed phenomenon.

The "internal resistance" of the cell decreases with increasing illumination (Fig. 4·4 *D*).

The spectral response extends from the x-ray region to about 1 to 1.2 microns in the infrared. The ordinary cell with a glass window exhibits a response curve as shown in Fig. 4·4 *E*.

The temperature coefficient is complex and is a function of the external circuit resistance (Fig. 4·4 *F*).

The average cell has an output of about 120 microamperes per lumen. The best high-vacuum cell of the photoemissive type yields 40 to 60 microamperes per lumen. For high levels of illumination, robust instruments such as micro- or milliammeters may be used with barrier-layer cells. In direct sunlight, 10 milliamperes has been obtained.

4·8 Vacuum phototubes. A highly evacuated tube with a composite cesium cathode may be taken as typical of this class. For a central anode tube we may discuss the characteristics in terms of Fig. 4·5.

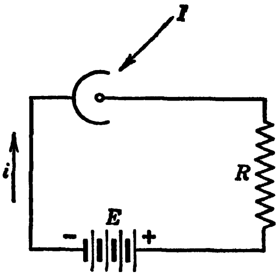

Fig. 4·5. Circuit employing vacuum photocell.

The tube is illuminated with light of intensity *I*, and under the influence of the applied potential, *E*, an electron current, *i*, flows through the load, *R*. Here *R* may represent an appropriate galvanometer or the input resistor of an amplifier. For constant illumination, current i will increase with applied potential E as shown in Fig. 4·6, indicating the saturation characteristic above a certain potential. For successively higher intensities similar curves are obtained (I_2, I_3, I_4, etc.). At very high intensities true saturation is not attained, and this is commonly observed with central anode tubes. Most commercial tubes are of this type. Their total output is greater than tubes of the central cathode type, which give saturation currents with nearly zero applied potential (7).

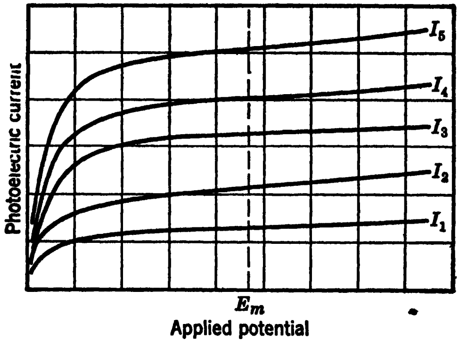

FIG. 4·6. Saturation characteristics of a central anode vacuum photocell.

It follows that for precise photometry some minimum potential, E_m, must be applied across the tube in order that saturation currents may be obtained for all intensities that are likely to be encountered. If we now plot the corresponding saturation currents against the corresponding light intensities, a straight line should result (Fig. 4·7). Returning to Fig. 4·5, we note that the potential across the tube is always less than E, by an amount equal to the voltage drop across R. In other words

$$E_c = E - Ri \qquad (4\cdot 1)$$

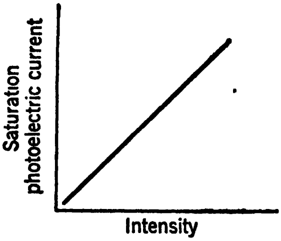

FIG. 4·7. Saturation current as a function of intensity.

This is especially important where amplification is used, for in this case the coupling resistor is usually chosen as high as possible ($R < 50$ megohms). Care must be exercised that the resulting Ri drop is not too large, thus bringing the potential across the tube to a value below the minimal E_m. A conservative choice in E will be 10 per cent in excess of E_m after the above correction has been calculated. An excessively high potential is undesirable because there can be no gain in response but only increasing contribution to leakage currents.

4·9 Special phototubes. Vacuum phototubes are almost invariably used with vacuum-tube amplifiers, but the equivalent of this amplification can be obtained within the envelope of the phototube itself in what is known as the photomultiplier tube. As a typical example, the RCA 931-A consists of a photocathode with nine additional electrodes or dynodes. The output electrode or anode completes the assembly. Primary photoelectrons from the cathode are accelerated at a potential between 50 and 100 volts to the first dynode, which emits secondary electrons which are then accelerated to the second dynode under similar conditions. This process is repeated at the successive dynodes, and beyond the ninth all secondary electrons are collected by the anode. Since two or more secondaries can be emitted at each dynode for every primary electron which strikes it, the total amplification will be given by the ninth power of the ratio of secondaries to primaries. This multiplier tube is no larger than an ordinary receiving tube and can achieve an amplification of several million fold. An additional advantage is the considerably higher signal-to-noise ratio when compared with a conventional phototube and amplifier. Although a low-power thousand-volt supply is required to supply the dynodes, several simple units for this purpose have been designed. The photomultiplier tube with its high-voltage supply is still more compact than a phototube-amplifier combination of comparable sensitivity. Complete characteristics and data for these tubes as well as the design of several power supplies are described in RCA tube manuals.

A recent development has provided means for improving the signal-to-noise ratio of an ordinary phototube (*8*) in which two coils, symmetrically disposed on either side of the tube and excited at 200 cycles, serve to modulate the photocurrent by magnetically distorting the photoelectron trajectories. The resultant photocurrent is passed through a tuned amplifier and read on an ordinary a-c voltmeter. The signal-to-noise ratio improvement arises from this modulation and largely minimizes errors due to leakage current.

The iconoscope and Orthicon tube are image-forming devices employed in television. They are designed for a vastly more complicated problem than ordinary photometry, but, from the fact that their light-sensitive mosaics are capable of delineating the complete details of a picture, they possess the inherent possibility of solving intricate photometric problems. For example, it is quite possible to project the beams from an almost unlimited number of photometric systems on a single iconoscope mosaic and measure each at high speed and in any desired sequence. The iconoscope or Orthicon seems to be the logical device for instantaneous spectrophotometry, in which the spectrophotometric curve is presented instantly and continuously on a cathode-ray tube. A cathode-ray spectrophotometer employing mechanical-optical scanning has been developed (*14*) which successfully delineates spectrophotometric curves, but any of the image-forming tubes would eliminate the necessarily cumbersome mechanical scanning.

4·10 Barrier-layer cells versus phototubes. It is important to keep in mind the relative advantages and limitations of each type of cell. There has been a tendency in some circles to regard the barrier-layer cell as vastly superior and simpler than the photoemissive type. This is by no means true, depending entirely upon the particular problem to be investigated. At very high levels of illumination the comparatively heavy currents furnished by a barrier-layer cell are impressive. However, in most photometric work the available radiant energy is feeble, especially if a monochromatic beam is employed. A rough comparison under these conditions will illustrate the point.

Suppose we consider a photronic cell of sensitivity 120 microamperes per lumen, which is receiving radiation of 10^{-6} lumen. The current will be 1.2×10^{-10} ampere. A good cell of the emissive type (40 microamperes per lumen) under the same conditions will deliver 4×10^{-11} ampere. If we use galvanometers of appropriate characteristics and a sensitivity of 10^{-10} ampere per millimeter, we shall obtain 1.2-mm. and 0.4-mm. deflection, respectively. In each case we would be able to detect light, but in no sense could it be measured accurately. With the photronic cell the dilemma is genuine because amplification is impossible, for, assuming the load resistance to be 5000 ohms, the available potential drop is only 0.6 microvolt. This is about the noise level of an amplifier, and while the effect would be detectable it could not be measured. In the emissive type, amplification with a single, stabilized FP-54 tube would solve the problem. A current of 25 microamperes could be obtained under the above conditions. Thus, full-scale deflection could be obtained on a 0 to 25 microammeter. Assuming 100 scale divisions, the photocurrent could be measured with better than 1 per cent precision.

The barrier-layer cell yields relatively large currents at a low potential. The internal resistance is low and decreases with increasing illumination. The emissive type yields smaller currents, but it has a very high resistance. Since the load should match the impedance of the source, it is readily seen why the emissive type is amenable to amplification.

4·11 Circuits. Vacuum phototubes are almost invariably used with some form of amplifier. The variety of vacuum tubes available for this and other purposes is very great, and the multiplicity of circuits employing such tubes is still greater. The earliest attempts to use amplifier tubes in conjunction with a phototube tended to achieve a high degree of stability and reliability; indeed special electrometer tubes were developed specifically for these and related applications. The present trend lies more in the direction of good circuit design, whereby less and less demand is placed upon the reproducibility of tube characteristics. Nevertheless, it is not surprising to find that modern tubes have been improved with respect to life, dependability, and reproducibility of characteristics several orders of magnitude better than their earlier prototypes. Notable advances in this respect are the RCA "Red Tube Series," which are designed for 10,000-hour service instead of the conventional 1000 hours (*20*), and the Victoreen miniature tubes which were specifically developed for wartime applications such as the proximity fuse, and instruments for nuclear research. The latter possess miniature characteristics requiring further amplification but have unusually high input impedance and almost complete immunity to leakage arising from humidity (*23*).

The principle of inverse feedback has revolutionized amplifier design for this and related applications. This method provides for the return of a definite fraction of the amplifier output to the input in a degenerative sense. If a large fraction of the output is so returned, it can be shown that the residual amplification becomes largely independent of all supply voltages and tube characteristics. It will be seen that this principle is essentially one in which the input signal is almost completely cancelled by an equal and opposite signal, and, as such, one is dealing with a null method without the intervention of an operator. The theory and practice of inverse feedback are described in several monographs, in all modern texts on radio and electronics, and in several thousand original papers (*1*, *3*).

As a typical example of the application of this method to photometry, reference is made to Fig. 4·8. The precision of this circuit is limited only by the output meter. A large-sized meter with an accuracy of 0.1 per cent of full scale has been used, and the output has also been registered by a recording potentiometer (*15*).

It might be inferred from the high degree of linearity which can be obtained from an inverse feedback amplifier that this is the most desirable relationship between photocurrent and output. Actually, a logarithmic response is somewhat more useful in photometry, and, aside from the obvious trick of providing the output meter with a logarithmic scale, a vacuum tube can be made to compute the logarithm. This has been done in a number of ways, one of the simplest of which is shown in Fig. 4·9. The logarithmic response can be achieved by providing a remote cut-off pentode (6SK7 or older 6D6) with a cathode resistor. With the correct choice of cathode resistor the plate current of the pentode is strictly proportional to the logarithm of the input voltage. This relationship holds over slightly more than 1 cycle of logarithms. Since the logarithmic relationship cannot extend to zero plate current, the normal component of the plate current must be balanced out. This is not a disadvantage because it provides the equivalent of a suppressed zero meter. If this logarithmic amplifier is used in con-

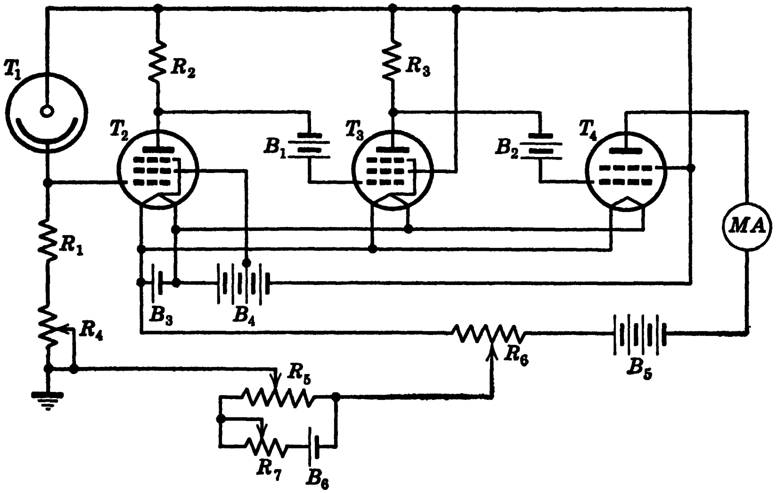

FIG. 4·8. Circuit for inverse feedback photometer.

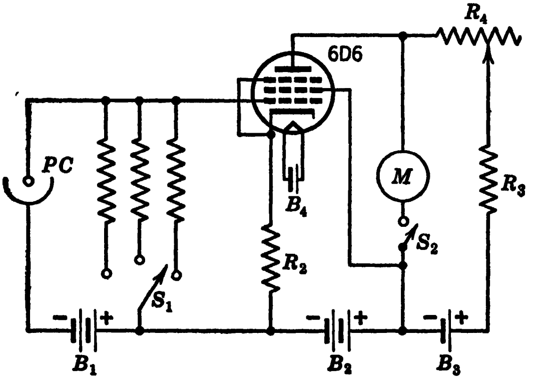

FIG. 4·9. Circuit with logarithmic response. Linear in concentration units.

junction with a phototube for photometry of solutions, it is obvious that the plate current meter can be calibrated directly in concentration units. Furthermore, for routine analyses one can provide a choice of phototube load resistors to accommodate the effective absorbance indexes of various substances. A practical photometer embodying these principles has been described in the literature (*16*), and a twin circuit for the automatic recording of absorbancy values has been developed (*19*).

It might be assumed that, when one has provided reliable amplification and suitable identification of the photocurrent on a meter, the essential requirements of a photometer have been met. It is interesting and important to note that the supposed elimination of fatigue by the substitution of photoelectric methods often results in the equally fatiguing chore of squinting at the pointer of a meter. This difficulty is easily eliminated in photometers employing optical compensation because the compensating diaphragm, wedge, or polarizer can be linked to a bold-faced dial or indicator. The electronic equivalent can also be achieved by an appropriate choice of circuit. For example, a phototube may be used to control the period of a relaxation oscillator, the pulses from which can be registered by suitable scaling and counting circuits. An alternate approach stores the photocurrent in a capacitor which is subsequently discharged through a resistor. The time required for the discharge of the capacitor between fixed potential limits is an integrated and logarithmic function of the total amount of light received by the phototube during a stated interval. This general principle is extensively employed in direct-reading spectrographs.

4·12 Balanced circuits with barrier-layer cells. *Circuit 1.* The circuit shown in Fig. 4·10 has often been used, but occasionally with the mistaken notion that it compensates for fluctuations in the light source. For the analysis of this and the following circuits we shall designate a common light source of intensity I which illuminates two essentially identical cells, 1 and 2. The light incident upon cell 1 passes through the sample of transmittance T. The respective photocurrents, i_1 and i_2, flow through the galvanometer in opposite directions. In this and the remaining cases, absolutely identical photocells are not necessary. It is merely assumed that each shall exhibit linear response over the prevailing light intensities and that they are initially adjusted (by virtue of position or the use of stops) for equal response. Slight differences in spectral response are usually of no importance, since most applications demand at least approximately monochromatic light. In this circuit the photocurrents in the two branches are

$$i_1 = kTI \quad \text{and} \quad i_2 = kI \qquad (4 \cdot 2)$$

where k is a constant. The net current through the galvanometer is

$$i_g = i_2 - i_1 = kI(1 - T) = kIA \tag{4·3}$$

where A is the absorptance.

With a constant source the galvanometer will indicate the absorptance directly. If the galvanometer sensitivity is adjusted by means of appropriate shunts, it may be set to read full scale (100 divisions) when $T = 0$, that is, when no light strikes cell 1. Under these conditions the scale will indicate per cent absorptance.

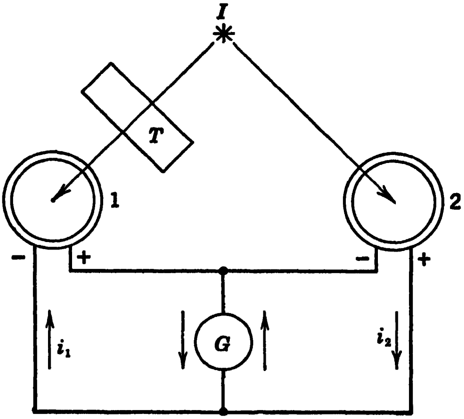

FIG. 4·10. Simple balanced circuit for barrier-layer cells.

We may seek the condition for compensation for source fluctuations by differentiating equation (4·3):

$$di_g = k\,dI - kT\,dI$$

Setting

$$\frac{di_g}{dI} = 0$$

we find that $kTI = kI$ or $T = 1$.

In other words, fluctuations in response will be a minimum when the transmittance of the sample is unity and the circuit has the doubtful distinction of perfect compensation when it is not in use. However, if optical compensation is used, the compensating feature can be retained.

Circuit 2 (Fig. 4·11). In this arrangement the two photocurrents, i_1 and i_2, flow through variable resistors R_1 and R_2. Values for the latter may be found such that the potential drops, E_1 and E_2, are equal, under which circumstances the galvanometer will show zero deflection. The circuit was suggested by Wilcox (*25*). No circuit analysis was given, and the following treatment, which has been confirmed by experiment, may be of interest.

As before

$$i_1 = kTI \quad \text{and} \quad E_1 = kTIR_1$$

$$i_2 = kI \quad \text{and} \quad E_2 = kIR_2$$

at balance $E_1 = E_2$ and therefore $kTIR_1 = kIR_2$ or

$$T = \frac{R_2}{R_1}$$

The transmittance of the sample is given by the ratio of the resistor settings necessary to establish balance. Various convenient arrangements are possible; for example, R_2 may be a Kohlrausch slide wire and R_1 an ordinary resistor of the radio "potentiometer" type. The slide wire is set at 100 with the sample removed, and balance is established with R_1. The sample is then introduced, and balance is re-established with the slide wire. The per cent transmittance is indicated directly on the slide wire. For the reasons before mentioned, both resistors should have very low values.

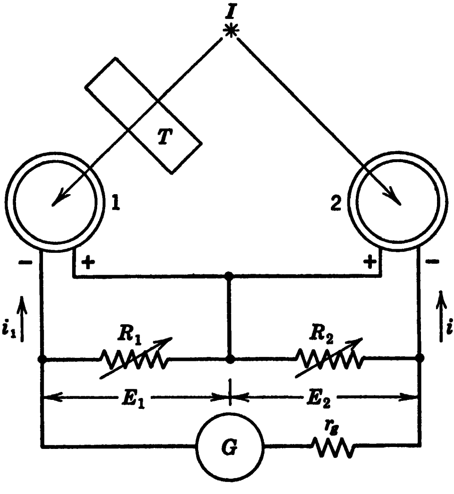

FIG. 4·11. Balanced circuit possessing compensation for source fluctuations.

Compensation for source variations is inherent in this circuit. The current through the galvanometer is

$$i_g = \frac{E_2 - E_1}{r_g} = \frac{kIR_2 - kTIR_1}{r_g} \tag{4·4}$$

Differentiating,

$$\frac{di_g}{dI} = \frac{kR_2}{r_g} - \frac{kTR_1}{r_g}$$

For

$$\frac{di_g}{dI} = 0$$

we get

$$T = \frac{R_2}{R_1}$$

From this result we see that the fluctuations due to source variations will be at a minimum when the resistors are set in the ratio of the transmittance, which is precisely what is done in the process of balancing the circuit. This may be confirmed experimentally in simple fashion. Writing equation (4·4) in increment form,

$$\Delta i_g = \frac{k\Delta I}{r_g}(R_2 - TR_1) = \frac{k\Delta IR_1}{r_g}\left(\frac{R_2}{R_1} - T\right) \qquad (4\cdot5)$$

Since R_2/R_1 is the transmittance, any value of R_2/R_1 may be designated as a virtual transmittance, T'. The last equation becomes:

$$\frac{\Delta i_g}{\Delta I} = \frac{kR_1}{r_g}(T' - T) = a\Delta T \qquad (4\cdot6)$$

where

$$a = \frac{kR_1}{r_g}$$

Figure 4·12 shows values obtained and plotted according to the above equation. In this test R_1 was held constant and the intensity of the common source was changed by known amounts, ΔI. The corresponding galvanometer deflections, Δi_g, were noted. Several values of $\Delta i_g/\Delta I$ were measured for different settings of R_2. In each case ΔT could be computed from the value of $R_2/R_1 - T$. These are the abscissas of Fig. 4·12, which shows that the fluctuations approach zero as the difference between R_2/R_1 and T (the true transmittance) approaches zero. The limiting case is most simply shown by setting R_2/R_1 equal to the true transmittance. The light source may now be dimmed almost to extinction without causing any perceptible galvanometer deflection.

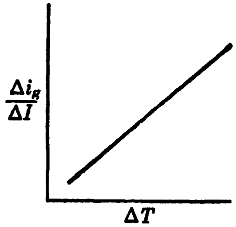

FIG. 4·12. Relationship illustrating equation (4·6).

Circuit 3 (Fig. 4·13). This arrangement with various modifications has been widely employed. Brice (*4*) has given an analysis of this and related circuits. Neglecting secondary factors, one may use this method to obtain transmittance values with good compensation for source variations. Figure 4·13 shows that the galvanometer may be considered as fed by two cells through universal shunts. In the right-hand branch, *R* may take the form of a low-resistance Kohlrausch slide wire.

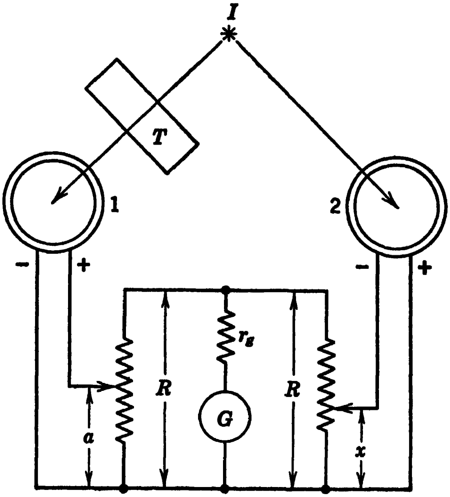

FIG. 4·13. Balanced circuit. Direct reading, with compensation for source fluctuations.

The opposing currents through the galvanometer may be written

$$i_{g_1} = kTI \frac{a}{R + r_g}$$

$$i_{g_2} = kI \frac{x}{R + r_g}$$

At balance these currents are equal; hence

$$x = Ta$$

In other words, x, the slide-wire setting, is directly proportional to the transmittance; indeed, if the initial adjustment is so arranged that, when $T = 1.00$, x is set at its maximum value, R (100 or 1000 scale divisions), and a is varied until balance is established, then all successive values of T will be given by x directly. Whenever electrical balance is established, the arrangement should be independent of source fluctuation, as may be seen by differentiating the expression for the net current through the galvanometer.

$$i_g = \frac{kIx}{R + r_g} - \frac{kTIa}{R + r_g} = cIx - cTIa \tag{4·7}$$

where

$$c = \frac{k}{R + r_g}$$

$$\frac{di_g}{dI} = cx - cTa$$

for

$$\frac{di_g}{dI} = 0 \quad x = Ta$$

Fluctuations due to source variations are therefore at a minimum when $x = Ta$, which is, however, the condition of balance as obtained during a measurement.

It should be emphasized that these considerations have neglected secondary factors, such as variation of the internal resistance of the cells, overshooting, and fatigue. Considerable care must be exercised in the choice of cells, the optimum level of illumination, and the correct magnitude for the resistance of the measuring current. Brice (*4*) reports deviations of no more than 0.1 to 0.2 division on a 0 to 100 scale, but this was achieved by a very careful study of the above factors. The paper should be consulted for detailed information on balanced circuits of this type.

4·13 Servomechanisms. There seems to be considerable opportunity for the improvement of filter photometers by applying to them relatively simple servomechanisms for the purpose of establishing photometric balance. A precedent already exists in the more elaborate photoelectric spectrophotometers, in which a state of photometric unbalance is detected by a phototube and the amplified photocurrent drives a motor which affects optical compensation in the reference beam. Whatever scheme is employed, it must be sufficiently simple and inexpensive to justify its use with a filter photometer. Numerous designs have been described which should be applicable to this problem (*1*, Section IV). The degree of simplification which can be tolerated depends on the use for which such an automatic filter photometer is intended. For example, if the object is to measure a system which is continually changing its optical properties, then a high-speed servo system possessing directional discrimination is required. These systems are not too simple or cheap. On the other hand, if it is required to measure a fixed sample with reasonable rapidity, it is possible to use one of the simpler relay servo systems which will seek or hunt out a condition of photometric balance resulting in zero signal. There are many examples of this class of servo systems where proportional speed response and directional discrimination are not primary requirements.

4·14 Light sources.* With the exception of mercury-arc sources, incandescent lamps are the most common light source in filter photometers. If flicker methods or twin-cell circuits are employed in the photometer, no particular degree of constancy is required of the light source. However, it should be noted that gross changes in the lamp current might well cause changes in the color temperature which would produce a change in the effective wavelength isolated by the filter system. For all other photometer circuits which demand long-term stability some means of accurate control is required. It is almost unnecessary to point out that measurement of the lamp voltage is a poor method of determining the correct operating point. The reason is the fact that the photocurrent from an incandescent lamp source varies between the third and fourth power of the lamp voltage. Specifically, a photometric precision of 0.1 per cent requires that a 6-volt lamp be held constant to within 1 or 2 millivolts. As far as automatic regulation is concerned, this is usually achieved by constant-current transformers, although none of these will provide regulation to the extent of better than 1 per cent. Electronic regulation of light sources has been worked out, and while these are necessarily quite complicated, it is possible to secure regulation to within 0.02 per cent. In this case a phototube is used to monitor the light source, and the output of this phototube controls power tube shunts (*15*).

Although it seems a minor point, it often happens that many difficulties arise in photometers from poor connections at the lamp socket, and it is good practice to solder leads directly to the bulb, unless the socket is known to be beyond suspicion in this respect. By the same token, any circuits which include a rheostat for the adjustment of lamp brilliance may be regarded as a potential source of trouble.

4·15 Filters.† Filters are available in great variety with respect both to transmittance characteristics and to size, shape, and mounting. Glass filters are to be preferred to those using colored gelatin film because they are more rugged and less susceptible to damage by heat.

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* See also Chapter 5, Section 5·9.

† See Chapter 5, Section 5·14, where filters are discussed more in detail from the viewpoint of "abridged" spectrophotometry.

The gelatin filters are more selective and come in a wider range of characteristics, but if they are used it is advisable to place them in the optical system as remote as possible from the light source. The advent of the new interference filters enables one to obtain very narrow bands. The total intensity obtainable with these filters is quite low, despite their high efficiency, and it is doubtful whether their advantages can be used with profit in barrier-layer cell photometers, although this is no particular deterrent to their use with phototube amplifier photometers.

The choice of proper filters for a given photometric problem requires a knowledge of the light-absorption characteristics of the system to be measured. Fortunately, the transmittance characteristics of commercially available filters can be obtained from the photometer or filter manufacturer, and the values for many of the standard filters are tabulated in handbooks. The selection of the correct filter is not quite as simple as this might imply, especially in a photoelectric photometer, because a given filter will be used with a light source and a selective phototube, and the characteristics of both phototube and source must be considered. For example, an incandescent light source will emit light having its maximum energy in the region of about 1 micron. If this is used in conjunction with a cesium phototube, the effective radiant energy may be shifted as much as 100 A. to the long wave side of the peak value exhibited by the filter itself. It is possible to recalculate the effective band pass of the filter in terms of the known energy distribution of the lamp and the selectivity curve of the phototube. For extensive survey work it has been profitable to calculate composite factors for phototube and source, from which the effective filter characteristics can be replotted. For an occasional measurement most investigators find it more convenient to test a few likely filters by empirical trial. In practically all cases, it is advisable to include a heat-absorbing filter to eliminate most of the near infrared, because many phototubes are sensitive in this region and quite a few filters do not absorb it; nor are the transmittance values in this region always tabulated in the literature.

4·16 Applications. Filter photometers provide very convenient and effective means for determining the light-absorptive capacity of systems with radiant energy of the band widths passed by the filters used. In general, the items so measurable are intensity of a given color and change in color. The latter includes the appearance and disappearance of a color and the transformation of one color to another. Such phenomena are the basis of important analytical applications, such as those mentioned below.

4·17 General quantitative determinations. The principal use of filter photometers is in the field of colorimetric chemical analysis. Collections of selected procedures for countless determinations are described in several monographs (*6, 10, 21, 22, 26, 27*). In addition, the periodical literature is carrying a steadily increasing number of such methods.

In many cases these directions include useful suggestions for the choice of filters. The recent literature on these methods almost invariably includes such details, but often the specification is some filter number peculiar to a particular instrument. Unless one is familiar with the manufacturer's designations, he will know neither the width of band passed by the filter mentioned nor its median wavelength (approximate center of the band). In some cases a specification such as S53 means that the median wavelength is about 530 mμ, and that, therefore, this filter would be appropriate for an aqueous permanganate solution which shows its maximum absorptive capacity at a wavelength near 525 mμ.

Also fluorimetric determinations may be placed under this heading. As noted in Chapter 1, certain substances, which are not themselves colored or do not react with color-forming reagents, show color when irradiated with ultraviolet radiant energy of suitable wavelength. The phenomenon is known as fluorescence.

Figure 4·14 illustrates the general arrangement of a two-cell filter photometer arranged for colorimetric measurement of such systems.

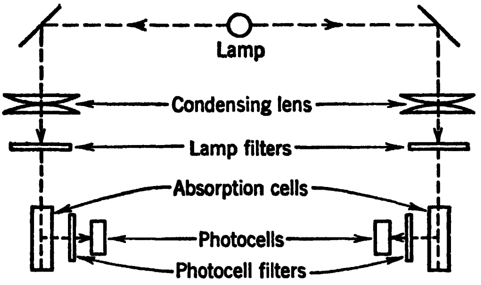

FIG. 4·14. Schematic arrangement of two-cell fluorimeter.

Compared to the photometers already discussed, the following important differences may be noted: (*a*) the source of radiant energy is frequently a mercury-arc lamp, certain lines from which provide the ultraviolet radiant energy; (*b*) the lamp filters pass only this ultraviolet radiant energy; (*c*) the photocell filters pass only visible radiant energy; and (*d*) the radiant energy measured emerges from the absorption cell in a direction at right angles to that of the incident beam.

Although fluorescing systems are in general not as useful analytically as ordinary colored systems, there are important applications, such as those for aluminum, riboflavin, and thiochrome.

Calibration curves. Mention may be made of several different methods of presenting data which are to serve as a calibration curve for a given instrument. It is assumed that the abscissa will be concentration of desired constituent. Depending upon the instrument, and also upon the method of using certain ones, the ordinate values for a series of concentrations may range from purely arbitrary dial readings to actual transmittancy (T_s) values. The latter are assumed here.

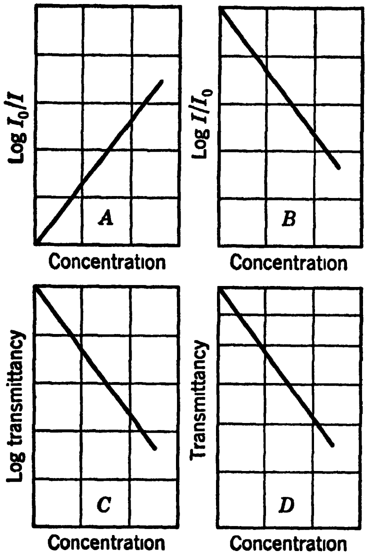

Fig. 4·15. Methods of plotting calibration curves.

Obviously, a curve coordinating transmittancy and concentration will serve as a working curve. However, many prefer to plot values in one of the ways illustrated in Fig. 4·15 (*11*). Since log T_s is a linear function of concentration for solutions conforming to Beer's law, the straight lines shown indicate such conformity.

It may be emphasized that each individual setting up a colorimetric method should establish his own calibration curve for his particular instrument, working under the same conditions to be used subsequently.

4·18 Photometric titrations. It is often profitable to study the change in transmittancy in a system as a function of the mole ratio of the reacting component. In many cases definite stoichiometric relationships can be revealed if the absorbancy is plotted against the mole ratio. The technique is reminiscent of conductance titrations, but in the optical case its value resides not in obtaining a single analytical result but in securing a better understanding of the mechanism of the reaction. This technique has been found useful in establishing the nature and composition of a light-absorbing substance (*12*, *13*). In the original investigation, the transmittancy values were followed with a recording potentiometer, and more recently it has been possible to use the logarithmic amplifier and record absorbancy values directly.

4·19 Automatic titrations. In the performance of automatic titrations, the methods of photoelectric photometry provide one of the possible means for detection of the end point (*18*). More recently available techniques permit the detection of the end point with great sharpness and certainty by using the change in transmittancy to operate a trigger circuit. The switching of the latter is so abrupt that the operation of the relay controlling the reagent-feed mechanism is very positive and reliable (*17*).

4·20 Inspection and supervision. Filter photometers of the photoelectric type are widely used in the recording of toxic gases and vapors, smoke, and other industrial hazards. A classical example is the detection of very minute traces of mercury vapor. This depends on the strong absorption which mercury vapor exhibits for its own resonance line, 2537 A. In most applications of this class ultraviolet radiant energy must be used, and except in rare instances no particular attempt is made to isolate any specific region of the spectrum, particularly if the presence of interfering substances is unlikely. Such equipment is usually calibrated empirically, and if the application is primarily a safety matter the results are automatically recorded and provision for sounding appropriate alarms is included.

4·21 Remote transmission of data. Photoelectric methods are well suited for the telemetering of optical data, and this technique may be expected to be of increasing importance, especially in industrial applications. In common with good telemetering practice, it is advisable to transmit the data as a change in frequency rather than in current or voltage. This practice renders the data immune to the effects of transmission line changes in resistance or temperature. The frequency shift is accomplished by causing the photocurrent to control a reactance tube, which in turn controls the frequency of an oscillator. The receiving element is simply an appropriately calibrated frequency meter, and its operation is independent of amplitude as long as the signal has been amplified sufficiently to overcome transmission line losses. Extensive references to this and related telemetering problems are to be found in a number of engineering textbooks.

4·22 Merits of filter photometers. Most of the different kinds of absorptimeters (here including comparimeters) were devised for more or less definite needs. When their utility has been demonstrated and has continued through the years, it is likely that such an instrument has met the need.

Filter photometers should be evaluated with respect to comparimeters and true spectrophotometers for the visual region. Although the merits of these two are presented in Chapters 3 and 5, respectively, it is well to repeat that most comparimeters are simple and economical, and that they serve very satisfactorily in many instances. No laboratory need be without some of the simplest of such equipment. Then we may anticipate Chapter 5 with at least the statement that the best spectrophotometers, operating on very narrow spectral band widths, are practically unique in their possibilities for use in determining mixtures of two or more colored constituents, such as the two chlorophylls, by employing simultaneous equations.

In general, the domain of filter photometers seems to lie between those for most comparimeters and all except the cheapest spectrophotometers. They are somewhat more expensive than comparimeters, but far less than any good spectrophotometer. The photoelectric photometer, of course, eliminates the fatigue and frequent uncertainty of visual matching, a characteristic considered by many as highly desirable. Reading the position of a needle on a dial seems enormously easier and simpler than color matching. Many industrialists consider the reliability better, especially under plant operating conditions. Individual measurements can be obtained rapidly by trained technicians. On the basis of the testimony of top industrial analysts, one seems justified in making the statement that filter photometric measurements are being extended rapidly. Some plants already report an amazing daily output.

Presumably any determination possible with a comparimeter can be adapted to a filter photometer. In the other direction, many determinations can be made with either a filter photometer or spectrophotometer. However, few conditions have been found favorable enough to use a filter photometer to determine two or more different components in the same sample, a practice increasingly common in spectrometry. This fact is explicable in terms of the relatively wide spectral bands passed by the filters in the simpler instruments. For this same reason the use of a few such filters does not enable one to determine a transmittancy-wavelength curve on a highly selective sample at all comparable to that obtainable with a spectrophotometer capable of operating on a narrow band width.

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5· SPECTROPHOTOMETERS:

Ultraviolet and Visible Regions

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A·INTRODUCTION

5·1 Definition. *Spectrophotometry* is a term that by practically universal usage is taken to refer to the relative measurement of radiant energy or radiant flux as a function of wavelength. By strict derivation the term might be restricted to such measurements made visually, but in actual practice the word has long been applied also to photographic and photoelectric methods of measurement in both the ultraviolet and the visible regions of the spectrum. More recently the term has been extended in application to cover also all such relative measurements in the infrared, throughout the region where the term *spectroradiometric* had heretofore been widely used because the detectors necessarily used were of the type conveniently covered by the word *radiometer*. The present extended usage is good, however, as it frees the word *spectroradiometric* for the absolute measurement of spectral distributions, as contrasted with the relative measurements for which the word *spectrophotometry* is so generally applied.

The significance of the word *relative* in this connection is that in spectrophotometry the measurements are always made relative to some standard. What this standard may be in any case depends on the type of measurement. For example, in spectral reflection measurements it may be a standard magnesium oxide surface; in transmission measurements it may be either the blank beam or a similar material or cell free of the absorbing constituent; in measuring the spectral distributions of light sources it may be a source of known spectral distribution. But in all cases what is measured is merely the ratio of two quantities, wavelength by wavelength, throughout the spectral range of interest.

5·2 Applications. The numerous applications of spectrophotometry may be grouped into three main categories:

1. Spectrophotometry may be used as a general research tool in investigations of the properties of materials. These investigations may relate to the relation between absorption and constitution or structure, the eye-protective qualities of glasses and other materials, the connection between absorption and fluorescence, the study and selection of optical filters, the bleaching or rancidity of vegetable oils, the fading of dyed materials, and many other types of work.

2. Spectrophotometry is of great usefulness in the rapid chemical analysis of solutions and other materials. Such measurements in the ultraviolet and visible spectral regions have long been valuable for this purpose, and in recent years similar measurements in the infrared have been found of immense importance, particularly in the petroleum industries. These applications are treated in other chapters. In the visible spectrum this type of analysis is often referred to as color analysis or chemical colorimetry, but such work should not be confused with the type of colorimetry referred to in paragraph 3.

Categories 1 and 2 are discussed in detail in Chapter 7.

3. Spectrophotometry is the fundamental basis of colorimetric analysis, standardization, and specification. This has become axiomatic in psychophysical colorimetry. It is the only fundamental means of analyzing a color for research or for other purposes. It is the only means of standardizing a color that is independent of material color "standards" (always of questionable permanence) and independent of abnormalities of color vision (existing even among so-called normal observers), and in this sense it is the basis for fundamental color specification. But while serving as the *basis* for color specification, a spectrophotometric curve (or table of data) is quite inadequate as a color specification in itself, particularly if colorimetric tolerances are desired as part of the specification. For adequate color specifications with tolerances the spectrophotometric data must be converted to some form of tristimulus specification by means of methods and data defining a standard observer, a standard coordinate system, and a standard illuminant. This is discussed in Chapter 9.

Only in the last application above is the spectral range of interest limited to the visible spectrum. In the first two applications any division of the spectrum into ultraviolet, visible, and infrared is essentially arbitrary and pointless. For example, the Beckman quartz photoelectric spectrophotometer covers the range from about 200 to about 1000 mμ, and its usefulness is in no wise limited as we enter or leave either end of the visible spectrum.

However, in the neighborhood of 1000 mμ (1 micron) there is a natural division, particularly as to instruments and methods. Neither the sensitized photographic plate nor the common types of photoelectric cells show much useful response above this wavelength. Radiometric methods, on the other hand, with the thermopile, bolometer, or other similar detector, which are universally used throughout most of the infrared, are but little used below 1 micron, particularly since the development of the cesium oxide and other phototubes of high sensitivity. Accordingly the techniques used above and below 1 micron are in general widely different, even though the type of measurement is often essentially the same. To a less extent this wavelength marks also a division in the types of materials investigated—for example, petroleum products above 1 micron, dyes and camouflage materials below 1 micron—but with many other materials such as glasses no such division is apparent.

The present chapter therefore covers the range from 200 mμ to 1000 mμ approximately. Spectrophotometry above 1000 mμ is treated in Chapter 8. Spectrophotometry below 200 mμ requires special equipment and technique, including measurement *in vacuo*, and little work in chemical analysis has been done in this region.

5·3 Historical. As already implied, the spectrophotometric methods to be considered here include the visual, the photographic,* and the photoelectric. However, consistent with the purposes of the treatise, the present chapter is not primarily an historical résumé of the subject. Rather, an effort has been made to make the treatment such as to have the greatest utility to present-day users of spectrophotometers. Naturally this results in considering spectrophotometers commercially available and widely used to a much greater extent than various spectrophotometers that have been individually designed and used in various laboratories but are not readily available to others.

It is accordingly desirable to list various published articles and books on spectrophotometry or related subjects which treat the matter historically, are of résumé nature, or give extensive bibliographies, so that those who wish may pursue the subject to any desired extent. Listed chronologically, with comments, these works are as follows, the complete reference being given at the end of the chapter:

Spectrophotometry, Report of Optical Society of America. Progress Committee for 1922–1923. A fairly complete treatment of the science as it existed at that time (*36*).

"The Use of the Photoelectric Cell in Spectrophotometry," K. S. Gibson. A résumé of the subject up to 1930 (*43*).

The Practice of Absorption Spectrophotometry, F. Twyman and C. B. Allsopp (1934). Special emphasis on photographic methods, but describes other instruments (*109*).

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* Details of photographic methods are given in Chapter 6.

"Current Advances in Photographic Photometry," G. R. Harrison (1934). Includes absorption spectrophotometry; contains references to work in extreme ultraviolet (*54*).

"Visual Spectrophotometry," K. S. Gibson. Describes visual instruments in common use in 1934 (*38*).

Measurement of Radiant Energy, edited by W. E. Forsythe and prepared under the direction of A. C. Hardy, Herbert E. Ives, and W. E. Forsythe (1937). Contains the following chapters of interest in spectrophotometry: I, "Fundamental Concepts and Radiation Laws" (Forsythe); II, "Sources of Radiant Energy" (Worthing); III, "Analysis of Radiation" (Adams); IV, "Spectrometric Instruments and Their Adjustments," (Kurtz); V, "The Spectrometer as an Optical Instrument" (Barnes); VII, "Measurement of Spectral Radiation by Means of the Photoelectric Tube" (Koller); VIII, "Measurement of Radiant Energy with Photographic Materials" (Jones); XI, "(Visual) Spectrophotometry" (Gibson); XIII, "Photometry" (Forsythe and Ives) (*26*).

"The Role of Spectrophotometry in Colorimetry," M. G. Mellon (1937). Of primary interest to the chemist (*82*).

"Photoelectric Methods in Analytical Chemistry," R. H. Müller (1939). Much of the information given would be useful in photoelectric spectrophotometry (*85*).

"Spectrophotometric Methods in Modern Analytical Chemistry," S. E. Q. Ashley (1939). Of primary interest to the chemist (*1*).

"Survey of Spectrophotometers," Kasson S. Gibson (1940). Discusses the measurement of spectral directional reflectance (*42*).

Chemical Spectroscopy, Wallace R. Brode (1943). Includes absorption spectrophotometry by visual, photographic, and photoelectric methods (*8*).

Spectrophotometry in Medicine, Ludwig Heilmeyer, translated by A. Jordan and T. L. Tippell (1943). Absorption spectrophotometry (*57*).

"Photoelectric Photometers: Their Properties, Use and Maintenance," J. S. Preston (1943). Much of the information given would be useful in photoelectric spectrophotometry (*93*).

"Photometry and Spectrophotometry," David L. Drabkin, a chapter in *Medical Physics* (1944). Absorption spectrophotometry, including filter photometry (*22*).

"Bibliography of Photoelectric Spectrophotometric Methods of Analysis for Inorganic Ions," J. W. Stillman (1945). Of primary interest to the chemist (*105*).

B·NOMENCLATURE—QUANTITIES MEASURED

In order to discuss the applications of spectrophotometry in the measurement of the spectral energy distribution of sources of light and radiant energy and in the measurement of the spectral transmission and reflection characteristics of materials, it is necessary to define clearly various terms used in such work and to assign symbols to these terms for convenient use.

Various groups and individuals have done this in the past, and various systems of terminology are thus available. Many of these are excellent in themselves and have served their purposes well. For example may be noted (1) in radiation, the definitions and symbols proposed and used in the book entitled *Measurement of Radiant Energy*, Chapter 1 (*26*); (2)

the photometric terminology prepared and issued by the Illuminating Engineering Society and the American Standards Association (*19*); and (3) various reports prepared by special committees of the Optical Society of America, dealing with colorimetry and spectrophotometry (*36*, *68*, *107*).

Naturally, however, such divergent groups and purposes have resulted in systems that are often inconsistent with one another. Furthermore, because of the extent and detail of some of these systems, recourse has often been made to Greek, bold-face, script, or other symbols that are inconvenient to use. Recently, however, a restricted set of terms for use in transmission and absorption measurements has appeared in *Letter Circular* LC-857 of the National Bureau of Standards (*86*). These terms were agreed upon by various members of the optics and chemistry groups of the Bureau and appear to be a satisfactory compromise of the various terms and symbols used in the past.

These transmission and absorption terms of LC-857 are given in this chapter, together with a few other terms useful in the spectrophotometry of light sources and reflecting materials.

5·4 Spectral energy distribution. In any fundamental consideration of the radiation characteristics of light sources or of sources emitting radiant energy at any wavelengths in the ultraviolet, visible, or infrared regions of the spectrum, one must consider the space and time variants involved in the radiation processes. This has been done to a greater or less extent in the nomenclatures and terminologies to which reference has just been made. In such we find, for example, definitions and terms for radiant energy, the time rate of flow of radiant energy, the solid-angular flux density in a given direction, the areal density of radiant flux from a source or on a surface, and the space density of radiant energy, together with the luminous analogs for the same terms.

For our purposes *radiant energy* is defined as energy traveling through space in the form of electromagnetic waves of various wavelengths. It is the entity that is emitted from radiators, that is reflected, refracted, absorbed, or transmitted by various objects or materials, and that is incident upon the various detectors, such as the thermopile, the photographic plate, the eye, and the phototube.

Radiant flux (power) is the time rate of flow of radiant energy.

In some cases the integrated amount of radiant energy is what is primarily measured by the detector; this is true of the photographic plate as customarily used and of the phototube if connected to certain electrometers or "photon-counters." In other cases the radiant flux is the quantity which the detector primarily indicates. This is true of the eye and of the phototube and thermopile with galvanometers or other devices as ordinarily used. It is often convenient, therefore, to consider the radiant flux as the entity to be measured or discussed.

In spectrophotometry, where at any frequency or wavelength only the ratio of two quantities is measured, it is usually possible and convenient to ignore the spatial and temporal variants of radiant energy and speak simply of the radiant energy itself, denoted graphically by an arrow and symbolically by the letter I. If I_0 refers to the standard at any wavelength and I to the unknown at the same wavelength, the quantity I is derived immediately from the measured ratio, I/I_0, and expressed in whatever units are used or known for I_0. In deriving the transmission terms given below, it will be noted that I and I_0 are no longer needed after the initial definitions of transmittance.

It is occasionally desirable, however, even in spectrophotometry, to refer to certain spatial variants of radiant flux, and these are accordingly defined here. The terminology of the Optical Society of America (*68*) is followed.

Radiant intensity of a source of radiant energy is defined as the radiant flux per unit solid angle in a given direction.

Radiance is the radiant intensity per unit projected area of the source.

Irradiance is the radiant flux per unit area incident upon a surface.

Before considering the transmission and reflection terms, a few other comments may be made.

Radiant energy and light. Light is the aspect of radiant energy of which a human observer is aware through the visual sensations arising from stimulation of the retina of the eye. Quantitatively, light is radiant energy evaluated in terms of the standard luminosity function (see Section 5·9). Usually radiant energy must have wavelengths between approximately 380 and 770 mμ if it is to have a luminous aspect. But it is not proper to state that radiant energy of wavelengths 380 to 770 mμ is synonymous with light. Consistent with this definition of light, the expressions *ultraviolet light* and *infrared light* are incorrect, and the word *light* is seldom used in this chapter even when visual spectrophotometry is under consideration.

In this connection may be noted the luminous analogs for the radiation terms just defined, starting with light or luminous energy, the standard luminosity function being the correlating factor in every case. Thus may be defined *luminous flux*, *luminous intensity* (candlepower), *luminance* (photometric brightness), and *illuminance* (illumination) (*19, 68*).

It is unfortunate that a short, single word for radiant energy, analogous to light, has not come into common use. Lacking such a word, we shall often use simply the word *energy*, where the context makes clear that radiant energy is meant. The use of *radiation* or *radiations* for radiant energy, as for example in the expression *radiations of various wavelengths*, is inconsistent with the modern usage that words ending in *-tion* (or *-sion*) are to be reserved for the designation of processes or for general and adjectival use.

Wavelength and frequency. The symbol for the wavelength of radiant energy is λ. The units in which wavelengths are expressed are restricted to those conventionally used for each spectral range; that is, millimicrons ($m\mu$) or angstroms (A.) for the ultraviolet and visible, and microns (μ) for the infrared. The symbol for frequency is ν. In place of true frequency a term designated as *wave number* is widely used, defined as $1/\lambda_{vac}$ (the unit of λ in this usage being usually the centimeter).

The relation between wavelength and frequency is

$$\nu = \frac{v}{\lambda} \tag{5·1}$$

where v is the speed of propagation of radiant energy. It may perhaps be emphasized that both v and λ change whenever radiant energy of frequency ν passes from a medium of one refractive index to a medium of different index. The frequency itself does not change, however, and is thus more fundamental than wavelength for theoretical studies.

5·5 Spectral transmission and absorption. In this section all the terms derived relate to the rectilinear transmission of radiant energy, and no consideration is given to radiant energy that may be deviated or scattered from its original direction. By far the greater amount of spectral transmission and absorption measurements are of this type, made upon materials such as liquids, solutions, glasses, or other nonscattering materials. If the material is turbid or translucent, the radiant energy scattered from its original direction must be considered. If this scattered energy is slight in amount and unavoidable, it may need consideration along with other sources of error. If the material is more highly diffusing, the measured transmittance will be importantly dependent upon the geometrical conditions of irradiation and reception; the situation is then analogous to that considered in some detail in Section 5·6 for reflection measurements.

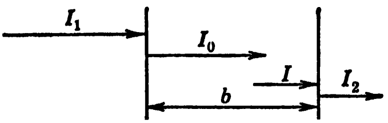

FIG. 5·1. Transmittance, T, is defined as the ratio I_2/I_1; internal transmittance, T_i, as the ratio I/I_0.

a. Terms relating to the rectilinear transmission of homogeneous radiant energy (radiant energy of a "single" frequency) through a homogeneous, isotropic, nonmetallic medium having plane, smooth, parallel surfaces.

In Fig. 5·1 let I_1 be the radiant energy incident upon the first surface of the sample, I_0 that entering the sample, I that incident upon the second surface, I_2 that leaving the sample.

$$T = \frac{I_2}{I_1} = \textit{transmittance} \text{ of the sample}$$

$$(100T = \text{percentage transmittance}) \qquad (5\cdot 2)$$

$$T_i = \frac{I}{I_0} = \textit{internal transmittance} \text{ of the sample} \qquad (5\cdot 3)$$

$$A_i = -\log_{10} T_i = \log_{10}\frac{1}{T_i} = \log_{10}\frac{I_0}{I} = \textit{absorbance} \text{ of the sample} \qquad (5\cdot 4)$$

Let b represent the length of absorbing path in the sample.

$$a_i = \frac{A_i}{b} = \textit{absorbance index} \text{ of the material} \qquad (5\cdot 5)$$

Other quantities derived from the above are occasionally used. These are $T_i^{1/b}$, which has been called *transmissivity*, $1 - T_i^{1/b}$, which has been called *absorptivity*, and $1 - T_i$, which has been called *absorptance*.

In the above, the thickness, b, or the unit of thickness should always be given. The temperature of the sample during the measurements should also be stated when it is a matter of importance.

b. Terms relating to the rectilinear transmission of homogeneous radiant energy through a homogeneous, isotropic, nonmetallic medium such as a liquid, mixture, solution, vapor, or gas confined between the windows of a cell.

Let T_{soln} be the (over-all) transmittance of a given cell containing a solution or homogeneous mixture of solids, liquids, vapors, or gases, of which the compound of interest is the solute or one constituent. Let T_{solv} be the (over-all) transmittance of the same or a duplicate cell containing pure solvent, or containing the same mixture in the same relative proportions minus the constituent of interest.

$$T_s = \frac{T_{\text{soln}}}{T_{\text{solv}}} = \textit{transmittancy} \text{ of the sample}$$

$$(100T_s = \text{percentage transmittancy}) \qquad (5\cdot 6)$$

[T_s does not precisely equal $(T_i)_{\text{soln}}/(T_i)_{\text{solv}}$. However, with end plates of refractive index not greater than 1.5, the error is negligible for most purposes.]

$$A_s = -\log_{10} T_s = \log_{10} \frac{1}{T_s} = \textit{absorbancy} \text{ of the sample} \qquad (5\cdot 7)$$

Let b represent the length of absorbing path between the boundary layers of the solution, and c the concentration of the solute or constituent of interest.

$$a_s = \frac{A_s}{bc} = \textit{absorbancy index} \text{ of the material} \qquad (5\cdot 8)$$

a_M is the *molar absorbancy index,* c being expressed in moles per liter and b in centimeters, and the temperature being given.

a_x is the partial absorbancy index, where x refers to the component in question.

In the above, the thickness and concentration, or the units, should always be given. The temperature of the sample during the measurements should also be given when it is a matter of importance.

5·6 Spectral reflection. Differing from the case of spectral transmission measurements, where the great majority of substances measured are not importantly scattering, and accordingly where only rectilinear transmission was considered, most materials whose spectral reflecting properties are of interest are more or less highly diffusing. Such materials include papers, textiles, structural glass, paints, ceramics, and the like, for which the analysis and specification of color are so important. However, the derivation of reflection terms herein is limited to those that are useful for spectrophotometry. Those who wish to consider the subject in greater detail should consult other papers on the subject (*63, 70, 80*).

Three quantities, designated as (total) reflectance, specular reflectance, and directional reflectance, are of primary importance in reflection spectrophotometry. Again we are dealing with homogeneous radiant energy in these definitions.

Reflectance is defined as the ratio of the radiant energy reflected from an object to that incident upon the object. Since no restriction is placed upon the directions of the reflected energy, this type of reflectance is often designated as the (total) reflectance. The (total) reflectance will vary with the direction or directions of the incident energy but cannot exceed unity.

Specular reflectance is the ratio of reflected to incident energy taken only in the direction of mirror reflection. It is the type of reflection given by perfectly polished glasses, metals, or other mirror-like surfaces, without scatter. The specular reflectance of an object will vary with the angle of incidence but, again, cannot exceed unity.

Directional reflectance [formerly called apparent reflectance (*80*)] may be defined loosely as the reflectance that an object appears to have in any direction or group of directions. It is the type of reflectance measured by most reflection spectrophotometers, and it is therefore of considerable importance that its meaning be fully understood, and the factors affecting its measurement be fully taken into account.

The concept is most easily defined in terms of a perfectly diffusing surface. With such a surface the ratio of the radiance to the irradiance (Section 5·4) is constant for all angles of reflection. Furthermore, the magnitude of this ratio for a perfectly diffusing surface is independent of the angle or angles of irradiation, provided only that the irradiance is constant. (If the radiant energy is in the visible spectrum, the perfectly diffusing surface will appear equally bright when viewed from any direction.)

The *directional reflectance* of a surface may accordingly be defined as the ratio of the radiance that the surface has under any specified set of irradiating and receiving conditions to the radiance that a perfectly reflecting, perfectly diffusing surface would have under the same conditions. Differing from the (total) reflectance or the specular reflectance, neither of which can exceed unity, the directional reflectance can have any value between zero and infinity.

Since no actual surfaces are perfectly diffusing, the values of directional reflectance for any sample will depend on the geometrical conditions of both the irradiating and the receiving beams. The importance of the precise specification of these geometrical conditions depends on the purpose of the measurements and on the degree of departure of the surface from the completely diffusing condition. Which of the numerous possible conditions to use also depends on the purpose of the measurements and, in spectrophotometry, is determined partially by the degree of conservation of radiant flux which various conditions will afford with diffusing materials.

Symbols for reflection terms have varied from group to group and author to author. For purposes of this chapter, (total) reflectance is designated by R. If it is desired to indicate the angle of incidence of the irradiating energy, a subscript may be added, as R_0 or R_{45}. If the irradiation is perfectly diffused, the symbol is R_D; if nearly diffused, the symbol is R_d.

Specular reflectance is often equivalent to (total) reflectance and the above symbols may be used. If it is desired to designate specifically that the reflectance is specular, the symbol R_S may be used, combined if necessary with the angle of irradiation, as R_{S45}.

Directional reflectance is designated by the symbol R with subscripts attached to indicate the directions of the respective irradiating and receiving beams, as $R_{0,45}$, $R_{D,60}$, $R_{45,45}$, $R_{d,0}$. In this connection it must be emphasized, however, that only the subscript D is specific. The angle subscripts refer to the directions of the axes of the respective beams. The angular spreads of these beams are also in many cases of considerable importance and should be given in any complete specification of irradiating or receiving conditions.

Reflectance, total or directional, has been defined above as a property of the object. In some cases, for example with papers and textiles, the reflectances and directional reflectances will vary with the thickness or number of layers of the material used in the measurement and may be affected by the reflectance of the backing used. If the reflecting properties characteristic of the material are desired, it is customary to measure the reflectance in such a thickness that any increase in thickness will not change the measured value, and the reflectance of the backing will accordingly have no effect on the value. Reflectance measured under this condition is termed *reflectivity* or *directional reflectivity* and is designated by the symbol r. Subscripts have the same meaning as when used with the symbol R.

An important relation in this connection is that known as the Helmholtz (*58*) reciprocal relation. As stated by McNicholas (*80*), "The loss in flux density which an infinitely narrow bundle of rays of definite wavelength and state of polarization undergoes on its path through any medium by reflection, refraction, absorption, and scattering is exactly equal to the loss in flux density suffered by a bundle of the same wavelength and polarization pursuing an exactly opposite path." By this relation it is shown that, for example,

$$R_{45,0} = R_{0,45} \tag{5·9}$$

and

$$R_{D,60} = R_{60} \tag{5·10}$$

In other words, the numerical value of directional reflectance obtained with irradiation at 45° and reception at 0° is the same as that obtained with irradiation at 0° and reception at 45° (and similarly for any other pair of angles); and the value of directional reflectance obtained with perfectly diffused irradiation and reception at 60° is the same as the value of (total) reflectance obtained with irradiation at 60° (and similarly for any other angle).

No commercial spectrophotometer has been designed to measure directly the spectral distribution of radiant energy scattered from diffusing materials, except as the measurement of spectral directional reflectance may be so considered. Those wishing to investigate the relation among scattering, reflectance, and opacity should consult a paper by Judd (*70*) and papers to which reference is made therein.

C·SPECTROMETRY

Every spectrophotometer includes at least five principal constituents: (1) a source of radiant energy adequately covering the spectrum range of interest for the measurements; (2) a spectral dispersing system by means of which radiant energy of any desired frequency or wavelength (in reality, usually a more or less narrow range of frequencies or wavelengths) may be isolated and used in the desired measurements; (3) a photometric system, by means of which the desired ratio of energies may be determined at the various wavelengths; (4) a suitable detector of radiant energy which may or may not be a direct part of the photometric system; and (5) a sample holder for transmission and reflection measurements.

Many of the details connected with these divisions of spectrophotometry are considered in connection with the various instruments discussed below. Many others, however, can best be treated in sections by themselves largely independent of the instruments (because they are pertinent to all instruments). The present sections accordingly deal with item (2), the spectral dispersing systems of spectrophotometers (Section 5·7) and with means for the wavelength calibration or check of such instruments (Section 5·8). The other items are considered in the sections on photometry (Sections 5·9 and 5·10).

5·7 Elements of spectrometer. Three methods are commonly used to effect spectral dispersion and isolation in the region from 200 to 1000 mμ. The dispersion may be obtained by means of refraction through a prism; or it may be obtained through the diffraction and interference associated with the fine-line diffraction grating; or there may be no dispersing system in the ordinary sense, the spectral isolation being effected by means of optical filters. The present section deals only with the prism and grating spectrometers. The use of spectral filters is considered in Section 5·14 and in Chapter 4.

a. Prism spectrometers. Glass or quartz prisms of 60° refracting angle, or equivalent prisms, have been widely, almost universally, used in prism spectrophotometers for the ultraviolet and/or visible. Two types are shown in Fig. 5·2.

One of the simplest forms is type *A*. Although simple, this illustrates all the essential elements of a spectrometer. These consist of the dispersing prism, *P*, with the 60° refracting angle at *r*, a collimator, *C*, carrying a lens and an entrance slit, S_C, and a telescope or second collimator, *T*, with lens and exit slit, S_T. The slits, S_C and S_T, and the refracting edge of the prism at *r* are parallel to each other and extend (usually vertically) at right angles to the plane of deviation and dispersion.

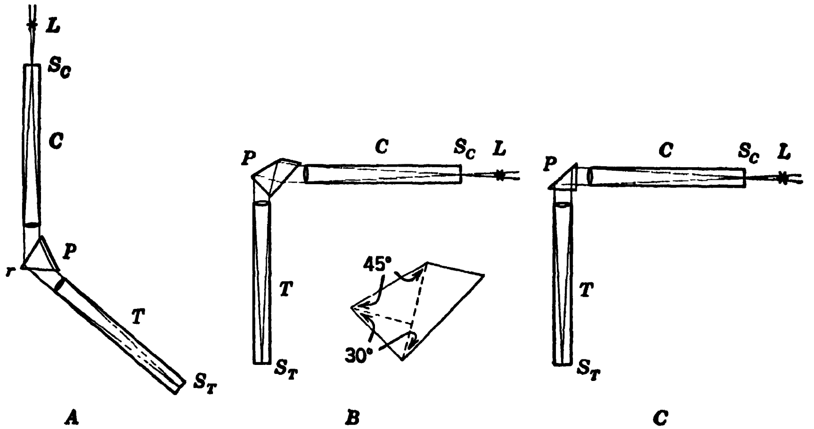

FIG. 5·2. Three types of spectrometers that have been used in spectrophotometers. *A* is the simple 60° prism type, *B* the constant-deviation 60° prism type, and *C* the transmission-grating type. In *C* the replica grating is placed on one of the right-angle faces of *P*. [Reproduced by permission from *J. Optical Soc. Am.*, **10**, 169 (1925). See ref. 36.]

By means of the collimator slit and lens, radiant energy from a suitable source, *L*, is formed into a beam, the various elements of which are approximately parallel. (These elements are closely parallel in the horizontal plane because of the narrowness of the slit, but in the vertical plane the direction of the elements may deviate from the horizontal by varying amounts determined by the length of the slit and the focal length of the lens.) This "parallel beam" undergoes dispersion by passage through the prism, the angle of refraction varying with the wavelength, and a spectrum image of S_C is formed in the plane of S_T.

By moving the telescope in the horizontal plane about a vertical axis located in the plane bisecting the angle *r*, the desired wavelengths may be selected at will for use in the observations. If the source, *L*, is one emitting radiant energy at certain wavelengths only (not a continuous spectrum), the images of S_C will appear as spectral "lines" characteristic of the particular source used. The width and length of these lines will vary with the width and length of S_C. If a source such as an incandescent-filament lamp is used (having a continuous spectrum), no lines are visible and the purity of the radiant energy isolated depends importantly on the widths of the slits, S_C and S_T.

Another type of spectrometer design is shown in *B* of Fig. 5·2. In this design the collimator and telescope are fixed at right angles to each other. Dispersion is effected by means of a constant-deviation prism. As shown in the enlarged diagram of *P*, this prism is equivalent to a 60° prism, consisting in effect of two 30° prisms separated by a right-angle totally reflecting prism. This type of spectrometer has two advantages over that shown in *A*: (1) both telescope and collimator are fixed in position, and change of wavelength is effected by rotating the prism, *P*; and (2) the rays accepted for measurement are closely at minimum deviation regardless of wavelength. Item (1) is of considerable advantage in connection with the construction of auxiliary equipment for use with the spectrometer; item (2) is less important in spectrophotometry. A disadvantage of this type of prism is the greater absorption within the dispersing prism caused by the increased length of path.

Modifications of these simple types of spectrometer are illustrated below in connection with certain of the spectrophotometers, such as the use of two spectrometers in series, and the use of a 30° dispersing prism with reflecting face. Still other designs of spectrometer, used principally in infrared work, are illustrated in Chapter 8 and in Refs. *26* and *68*.

b. Grating spectrometers. The theory and use of diffraction gratings may be found in textbooks on optics. Concave reflecting gratings and plane transmission gratings have both been used in spectrophotometry, particularly in the form of replicas. A simple form of spectrometer of this type is shown in *C* of Fig. 5·2. The grating is cemented onto one of the faces of the right-angle glass prism, *P*. The prism also serves to redirect the beam as shown, giving a convenient form of constant-deviation spectrometer. Wavelength is varied by turning *P*.

c. Transmission and dispersion of prism and grating instruments. In most applications the spectral transmittance of a spectrophotometer is of little importance so long as it transmits sufficiently to give adequate sensitivity. However, all prism material will absorb strongly at either end of its normal usable range, and it is a matter of some interest to know what the transmittance of certain typical prism or grating instruments may be.

In Fig. 5·3 are shown various transmittance curves of certain dispersing prisms and prism instruments, curves 1 to 4. Most of the absorption in the visible spectrum in a prism spectrophotometer is caused by the prism itself. The selectivity depends of course on the type of glass and the length of path in the prism. It is obvious from this illustration that any constant-deviation glass instrument cannot be used much below 400 mμ.

An additional factor limiting the transmission of glass instruments in the ultraviolet is the Canada balsam used in the achromatic lenses of the collimators. A thin film of this material absorbs strongly below 360 mμ.

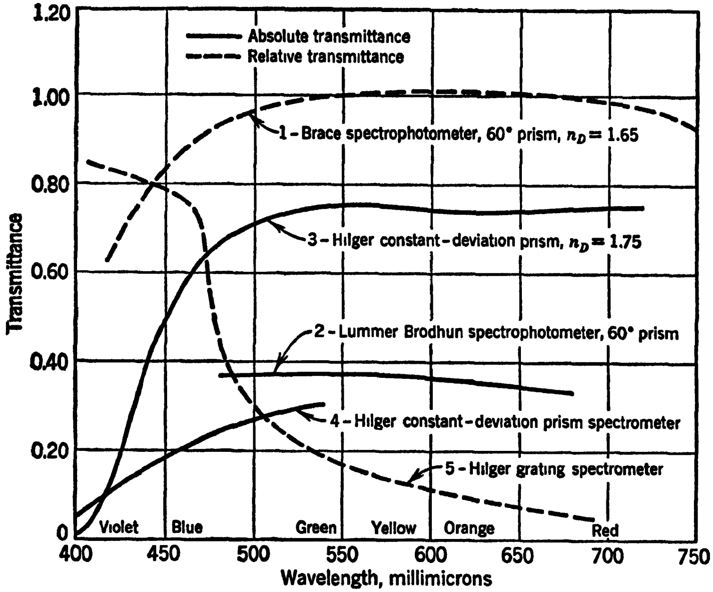

FIG. 5·3. Spectral transmittance curves of various glass prisms or prism instruments and of a grating spectrometer. [Reproduced by permission from *J. Optical Soc. Am.*, **10,** 169 (1925). See ref. 36.]

In Fig. 5·3, curve 5, is shown the relative transmittance curve of a replica-grating spectrometer, and in Fig. 5·4, curves 1 and 2, the absolute transmittance curves of a grating monochromator in the first and second order as determined by French, Rabideau, and Holt (*30*). In actual use, of course, for any one setting of the grating, the two orders do not overlap as shown.

Spectral transmittance curves for quartz-prism monochromators have been determined in the ultraviolet—for a crystalline-quartz two-prism instrument by Forsythe and Barnes (*28*), and for a fused-quartz single-prism instrument by Kay and Barrett (*72*). These are shown in Fig. 5·4, curves 3 and 4, together with the dispersion curve for a quartz-prism spectrophotometer, curve 5. The dispersion data are taken from one of the Beckman bulletins (*88*).

In Fig. 5·5 are shown (1) a dispersion curve, dashed line, of a typical glass-prism spectrophotometer, (2) at top and bottom, the amounts of pure spectrum transmitted by this particular instrument for an ocular slit set at 0.20-mm. width, in terms of frequency and wavelength, and (3) wavelength and frequency calibration curves (which are considered further below). In Fig. 5·5 the dispersion is plotted as angular scale divisions per millimicron; in Fig. 5·4, as millimicrons per millimeter of slit width. For comparison it may be noted that at 525 mμ the glass-prism instrument transmits about 4 mμ of pure spectrum and the quartz-prism instrument about 5 mμ of pure spectrum, for an exit slit set at 0.20 mm.

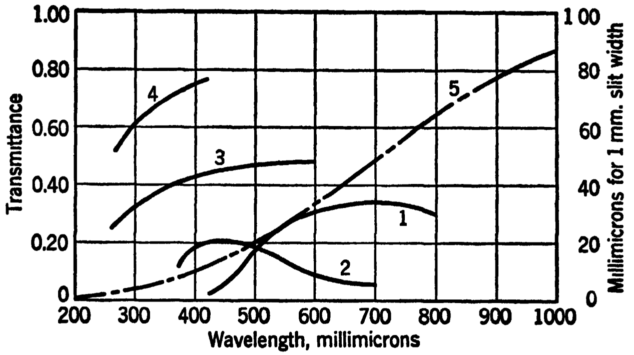

FIG. 5·4. Spectral transmittance curves of certain spectrometers: curves 1 and 2, a grating spectrometer in the first and second orders, respectively; curve 3, a two-prism crystalline-quartz monochromator; curve 4, a single-prism fused-quartz monochromator. Curve 5 is the dispersion curve of a quartz spectrophotometer. [Data for curves 1 and 2 taken from French, Rabideau, and Holt (*30*), for curve 3 from Forsythe and Barnes (*28*), for curve 4 from Kay and Barrett (*72*), and for curve 5 from one of the Beckman bulletins (*88*).]

Figures 5·4 and 5·5 illustrate how the extent of pure spectrum transmitted by an exit slit of constant mechanical width varies through the spectrum with a prism instrument. At the longer wavelengths the wavelength range thus transmitted is several times that transmitted at the shorter wavelengths. In general, therefore, the longer the wavelength, the smaller must be the mechanical width of slits to obtain the same purity of spectrum band for use in the measurements. Accordingly, if one wishes to maintain a constant spectral purity at the various wavelengths, he must continually change the slit widths mechanically or use some form of cam arrangement that will do this automatically.

In a grating instrument, because of the normal dispersion, the amount of spectrum transmitted by slits of constant mechanical width is unchanging through the spectrum.

In the above, reference has been made to the amount of "pure spectrum" transmitted by the exit slit of a spectrophotometer, and this is the customary way of expressing the slit widths of such instruments. By a pure spectrum is meant one that is formed with the entrance slit set at an infinitesimal width. In order to secure sufficient spectral energy for practical use, however, the entrance slit must be set at finite width, and it has become customary to set the entrance and exit slits at the same width (Section 5·20). With an incandescent or other continuous-spectrum source, however, this results in an extension of the wavelength range transmitted by the exit slit beyond that for a pure spectrum.

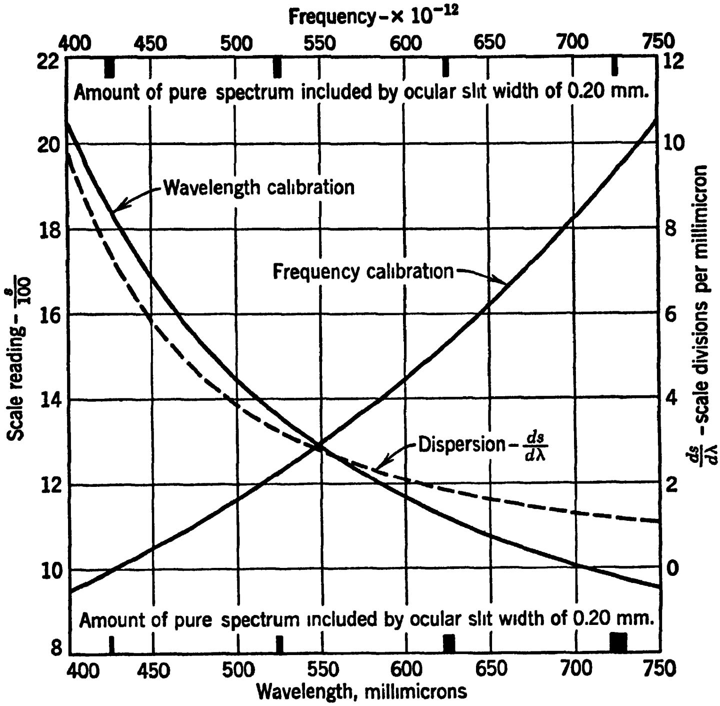

FIG. 5·5. Wavelength, frequency, and dispersion characteristics of a typical glass-prism spectrophotometer not having a direct-reading wavelength scale. [Reproduced by permission from *J. Optical Soc. Am.*, **10**, 169 (1925). See ref. 36.]

This is illustrated schematically in Fig. 5·6. In this figure, *b* represents the exit-slit width, *a* the entrance-slit width, both expressed in wavelength units. In *A*, slit *a* is very narrow and *b* is set at any nominal width. In *B* and *C*, slit *b* has been left unchanged and slit *a* widened to equal 0.5*b* and *b*, respectively, as shown. Relative amounts of energy transmitted by slit *b* are shown vertically as a function of wavelength. The important thing to note is the additional wavelength range that is transmitted by *b* as *a* is widened. This is indicated by the triangle-shaped areas in *B* and *C* that are shown on either side beyond the extent of *b*. When the two slits are equal, case *C*, the total range of wavelengths transmitted by *b* is 2*b*, although the fraction of energy transmitted by *b* at these additional wavelengths is only one-fourth of the total and is weighted in accordance with the triangular outline. (For a prismatic spectrum the triangle of Fig. 5·6 *C* is not perfectly symmetrical because of the variable dispersion, but the differences are negligible with any reasonable slit width.)

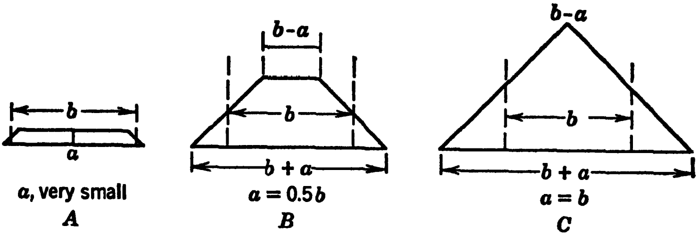

FIG. 5·6. Relative distributions of energy transmitted by a spectrophotometer at any wavelength as the entrance-slit width, *a*, is varied relative to the exit-slit width, *b*. Spectrophotometer slits are usually set at equal widths, illustrated in *C*, and the extent of spectrum transmitted is notably greater than that expressed in terms of either slit alone, as in *A*. It is customary to designate slit widths in terms of *b* alone and to designate them as nominal slit widths, and this is done in the present chapter; but it is well to remember that the effective slit width is always somewhat greater than *b*, the nominal slit width.

5·8 Wavelength calibration. The older spectrophotometers were usually designed with an arbitrary angular or linear scale for the wavelength setting. Such a scale had to be calibrated in terms of wavelengths or frequencies, somewhat as illustrated in Fig. 5·5 by the respective continuous curves. The wavelength calibration curve can be more or less represented by various dispersion formulas, such as

$$\frac{1}{s - s_0} = A + B\lambda \qquad (5\cdot 11)$$

or

$$\frac{1}{s - 1} = A + B\lambda^2 \qquad (5\cdot 12)$$

wherein s is the scale reading, λ is the wavelength, and A, B, and s_0 are constants to be determined. In general, however, these formulas will not give an adequate calibration except over a very short wavelength range. Their principal advantage is, perhaps, to facilitate calibration by enabling Δs, instead of s, to be plotted as a function of wavelength.

Most of the present-day spectrophotometers have a direct-reading wavelength scale; that is, the scale, instead of being divided in uniform linear or circular measure, is divided and engraved directly in millimicrons. This greatly facilitates setting the instrument at any desired wavelength. The accuracy of many of these direct-reading wavelength scales is remarkably good, when put in the best average adjustment, considering the difficulties of quantity production of such scales. When so adjusted, it is not uncommon to find them in error by not more than 1 mμ throughout the ultraviolet and visible spectrum. However, if one wishes the uncertainties in his wavelength settings to be of the order of 0.1 mμ, a careful check of these direct-reading scales is necessary.

The best procedure to use for checking the wavelength scale of a spectrophotometer depends on whether it is a nonrecording or a recording spectrophotometer.

a. For nonrecording spectrophotometers. The best procedure for checking the wavelength scale of a nonrecording spectrophotometer is by direct use of a source of radiant energy having spectral lines of suitable intensity and adequately spaced throughout the spectral range of interest. Various sources are available and can be recommended for such purpose. How many sources, or how many wavelengths, to use in such a calibration depends, of course, on the desires of the individual investigator.

In this connection it should be noted that the number of significant figures of importance in spectrophotometry (including "absorption spectroscopy") is of a different order of magnitude than that used in emission spectroscopy or in standard wavelength tables. In the visible spectrum with the usual type of spectrophotometer it seems impossible to maintain the wavelength calibration with uncertainties less than about 0.1 mμ. Although the uncertainty may be less in the ultraviolet with a prism instrument, no purpose seems served in giving standard wavelengths to better than 0.01 mμ for spectrophotometric calibration.

Two of the most suitable sources for wavelength calibration of spectrophotometers are the quartz- or glass-mercury arc and the ordinary helium vacuum tube in glass. If the range from 220 to 1014 mμ is of interest, the quartz-mercury arc is by far the best single source. A glass-mercury arc gives the same wavelengths as the quartz arc above 300 mμ.

The helium lines are especially well placed for wavelength calibration in the visible spectrum, and the strong lines at 388 and 1083 mμ are also often very useful. Many other sources, flame or arc, are available for visual wavelength calibration (*36*, *55*), but most of them are too instable for accurate calibration with a photoelectric detector.

These same sources and many others are also useful for the wavelength calibration of spectrographs used in photographic spectrophotometry. Between 200 and 400 mμ the series of doublets obtained from the aluminum spark in air is very useful because they are so readily recognized.

The wavelengths characteristic of the above sources, with a few others that have proved useful, are noted in Table 5·1. All values are consistent with those published in the M.I.T. wavelength tables (*55*). Not all the lines for any of the sources are given, but only those that are considered especially suitable for the purpose. Furthermore, not even all those listed for any one source may be suitable for any one particular instrument. Take the mercury arc in quartz, for example. All the lines listed (and still others) could be used for wavelength calibration of a photographic spectrophotometer over the range of sensitivity of the plate used. And all the lines from 404.7 to 690.7 mμ can be used for visual calibration of a spectrophotometer. But not all the lines are suitable for calibration of a photoelectric instrument, and those that prove adequate will depend on the sensitivity and slit widths characteristic of any particular instrument. One must be very careful that other lines are not included, in addition to the one on which the settings are supposedly being made, of sufficient intensity to affect the wavelength setting.

Special attention should perhaps be called to the use of a cesium arc at 852.1 and 894.3 mμ (*5*). From Table 5·1 it is apparent that suitable lines between 706.5 and 1014.0 mμ are otherwise mostly unavailable, particularly from steady sources necessary or desirable in the calibration of photoelectric spectrophotometers. The neon discharge tube gives many lines between 750 and 1000 mμ (*55*), but they have not been found satisfactory in the calibration of photoelectric spectrophotometers. In the orange and red the neon lines serve for visual calibration, and many of these can be used to calibrate photoelectric spectrophotometers (*44*) if the sensitivity is such that very narrow slits can be employed. The relative intensities given (*33*) will help in case of overlapping.

The best technique to use in wavelength calibration of nonrecording spectrophotometers, given a suitable source, will vary from instrument to instrument and method to method. A few general principles can be given here, however.

TABLE 5·1 SOURCES AND WAVELENGTHS SUITABLE FOR THE CALIBRATION OF SPECTROPHOTOMETERS

(WAVELENGTHS GIVEN IN MILLIMICRONS)

Mercury Arc in Quartz (Same Wavelengths in Glass above 300 mμ)		*Aluminum Spark in Air*	*Helium Discharge Tube in Glass*	*Hydrogen Sodium Cesium Arcs*	*Neon Discharge Tube*	
Wavelength	Remarks	Wavelength	Wavelength		Wave-length	Relative Intensity
205.29		216.88	318.77	H 434.05	585.25	5.2
222.47		217.40	361.36	H 486.13	588.19	3.9
223.41			363.42	Na 589.00	594.48	7.7
225.88		220.46	370.50	Na 589.59	597.55	1.7
230.21		221.00	381.96	H 656.28	603 00	2.3
232.32			388.86	Cs 852 11	607.43	7.8
235.25		226 35	396.47	Cs 894.35	609.62	13 0
237.83		226.91	402.62		614.31	24.6
239.94	1		412.08		616.36	5.9
239.97		236.71	414.38		621.73	3.9
244.69		237.21	438.79		626.65	11.4
246.41		237.31	443.75		630.48	4.3
248.20	2	237.34	447.15		633.44	19.9
248.27		237.84	471.31		638.30	22.8
248.38			492.19		640.22	100.0
253.48	3	256.80	501.57		650.65	38.6
253.65		257.51	504.77		653.29	7.9
257.63		257.54	587.56		659.90	11.8
260.32			667.81		667.83	23.4
265.20	4	263.16	706.52		671.70	14.0
265.37			728.13		692.95	23.0
265.51		265.25	1083.0		702.41	2.–
269.95		266 01			703.24	44.9
275.28					705.91	.. .
275.97		281.62			717.39	4.9
280.35	5				724.52	17.3
280.45		308.22			743.89	4.5
284.78		309.27			748.89	. . .
289.36					753.58	....
292.54		358.69			754.40	.....
296.73						
302.15	6	394.40				
302.35		396.15				
302.56						
302.75						
312.57						
313.15	7					
313.18						
334.15						
349 28						
365.01	8					
365 48						
366.29						
366.33						
390.64						
398.40						
400 63						
404.66						
407.78						
435.83						
491.60						
546.07						
576.96	9					
579.07						
623.44						
671.62						
690.72						
1014.0						
1128.7						

1. A value of 239.95 is recommended for the unresolved pair.

2. A value of 248.3 is recommended when the three lines are unresolved.

3. The intensity of 253.48 is negligible compared to that of 253.65. The latter value should be used when the lines are unresolved.

4. The 265.20 line is somewhat stronger than the others, and a value of 265.3 is recommended when the three lines are unresolved.

5. These two lines are of approximately the same intensity, and a value of 280.40 is recommended for the unresolved pair.

6. The two shorter lines are considerably stronger than the other two. It is probable that a value of 302.25 should be used for the unresolved lines.

7 A value of 313.16 is recommended for the unresolved pair.

8 With the arc used on the Beckman spectrophotometer the ratio of intensities for 365.01 365.48:366.33 is 100·48 36, approximately. The intensity of the 366.29 line appears negligible relative to that of 366 33.

9. These two lines are of approximately the same intensity, and a value of 578.0 is recommended for the unresolved pair.

In photographic spectrophotometry it usually is sufficient to photograph a known spectrum at the top and bottom of the plate, unless the source used for the absorption spectra itself carries such known reference lines. A few of these reference lines will then serve to correlate the particular plate with whatever complete calibration curve has previously been established by more extensive measurements with the various sources.

On visual and photoelectric nonrecording spectrophotometers, for highest precision, it usually is necessary to have a basic reference line to which all the other wavelengths are compared by direct check. At the National Bureau of Standards the mercury yellow lines have proved most suitable on the König-Martens visual spectrophotometer (*81*). At the slit widths used, the overlapping of the two lines gives a central brighter "line" taken as 578.0 mμ with a luminous background against which the slit jaws are readily seen. A luminous background, or slight illumination of the ocular slit, always facilitates calibration when an eyepiece is used. Visual calibration without an eyepiece is usually less precise unless very narrow slits are used.

Incidentally, the curvature of spectrum lines produced by a prism with straight entrance slit is readily observed in visual calibration with an eyepiece. This curvature is caused by the fact that the ray from the center of the slit is passed at less deviation than the rays from other parts of the slit. Some instruments are made with one or the other of the slits curved to remedy this defect. If both slits are straight, there is an effective broadening of the slit width and, visually, a greater uncertainty of wavelength calibration unless the lengths are kept rather small.

Two techniques have been used at the National Bureau of Standards in the calibration of nonrecording photoelectric spectrophotometers. On the Gibson spectrophotometer (*35*) the slits are always 0.1 mm. or greater and the most reliable calibration is obtained by plotting galvanometer deflections at closely adjacent wavelengths. This is illustrated in Fig. 5·7. The most probable value for the wavelength reading is given by the intersection of the two straight lines as shown, the correction being given by the difference between this value and the true wavelength.

On the Beckman spectrophotometer the same method has been used (*14*), but at the Bureau it has seemed preferable, and is much more rapid, to calibrate with a slit of 0.02 or 0.03 mm. and note the farthest throw to the left (of the rapidly responding needle) as the wavelength dial is slowly turned. The most suitable reference line on two of the Bureau's instruments has proved to be the mercury green line (*44*).

b. For recording spectrophotometers. The initial wavelength calibration of a recording spectrophotometer, such as the manufacturer must carry out in connection with cutting his cams or preparing his reading scale, is not here considered, but only the check of such a calibration by the user of the instrument.

The user can, of course, follow the procedure prescribed above for checking the wavelength calibration of nonrecording spectrophotometers. However, there are two important reasons for following a different procedure for recording spectrophotometers. For such instruments it is desirable to have a "dynamic calibration," one that is made with the instrument operating. It is further desirable in many kinds of work to have this calibration appear on the graph sheet so that worries connected with positioning of the sheet, expansion or contraction of the paper with humidity or temperature, or instrumental variations can be eliminated.

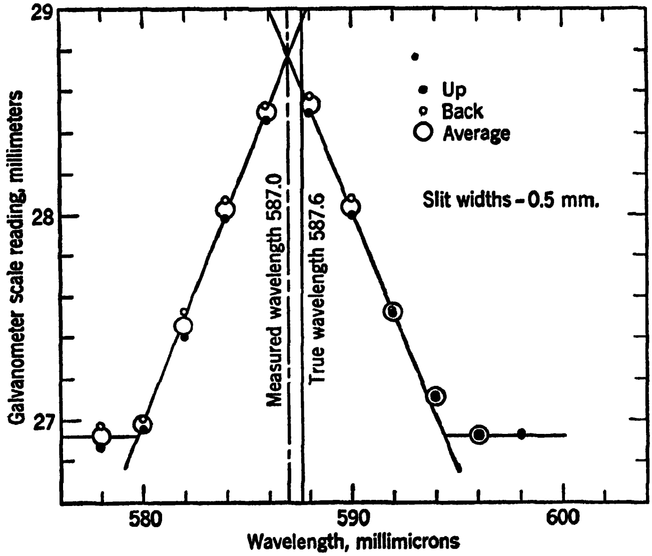

FIG. 5·7. One method of checking the wavelength calibration of a direct-reading nonrecording photoelectric spectrophotometer. Zero deflection of the galvanometer happened to be at 26.92 mm. in this illustration.

Wavelength calibrations of this kind can be made if a material is available having a number of strong and narrow absorption or transmission bands suitably spaced over the spectral range of interest. Two materials have been used or suggested for this purpose: (1) didymium glasses have been used for many years at the National Bureau of Standards (*45*), and many have been calibrated and issued to others for similar use, (2) quartz-Polaroid combinations have been proposed (*11*) and may prove useful for such work.

The use of a didymium glass in this manner would not in general be accurate unless it is calibrated at closely the same slit widths at which it is to be employed. Most of the absorption bands that are usable for the purpose are multiple bands, and the wavelengths of maximum absorption often depend on the slit widths. This is illustrated in Fig. 5·8, where the curves recorded on a General Electric spectrophotometer (*52,*

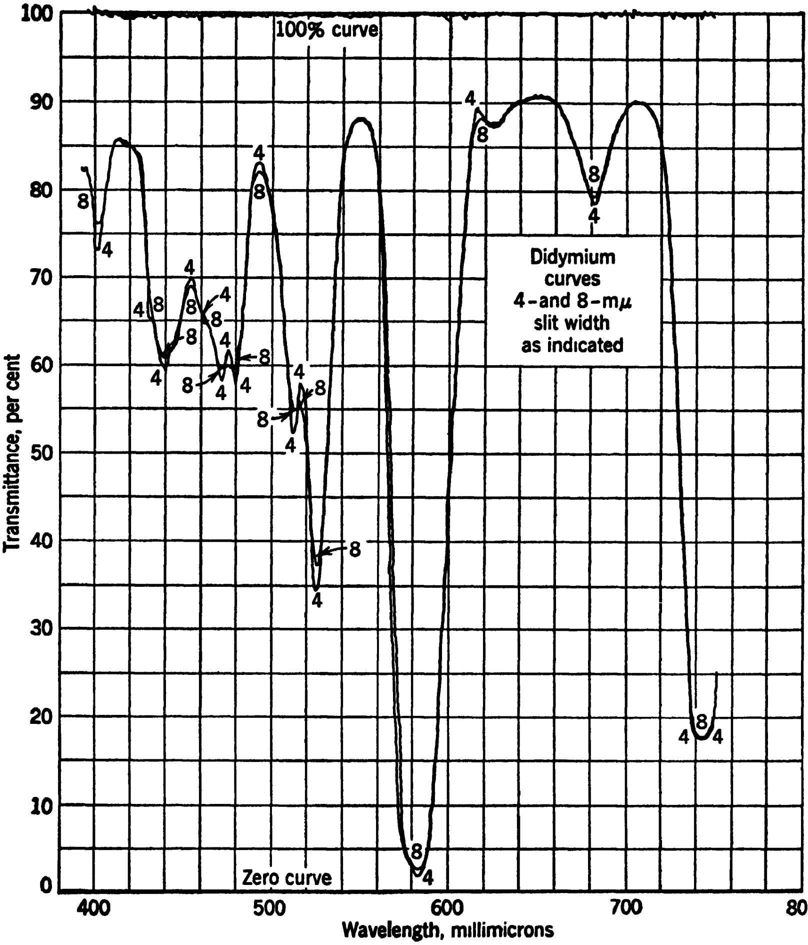

Fig. 5·8. Spectral transmittance curves of a didymium glass recorded on a General Electric photoelectric spectrophotometer at the National Bureau of Standards with nominal slit widths equivalent to 4 and 8 mμ of spectrum, as shown. For the two slit widths, note (1) shifts of the wavelengths of maximum absorption for the 480- and 513-mμ bands and (2) differences in recorded transmittances at all regions of sharp curvature.

53, 84) at the National Bureau of Standards (*45*) are shown for slits equivalent in width to 4 and 8 mμ of spectrum approximately.

The N.B.S. didymium glass standards were carefully calibrated by point-by-point measurements on the König-Martens visual and Gibson photoelectric spectrophotometers with slit widths approximating the 4-, 8-, 10-, and 20-mμ slits used on the N.B.S. General Electric spectrophotometers. Some of these values have been published (*74*). Although these initial calibrations were tedious and time-consuming, the time and worry eliminated by the use of such standards are enormous. Once calibrated, it has been very simple to derive corresponding values for glasses submitted for calibration by recording their transmittance curves on a sheet along with the curves for the N.B.S. calibrated standards.

The most suitable didymium glass for the purpose, considering type of curve and availability, is a Corning 5120 glass of 3.0-mm. thickness. Although it is not known how much the wavelengths of maximum absorption of this 5120 glass may vary from melt to melt, there has never been any certain variation among the samples tested. For much work it is probably safe to use the values given in Table 5·2.

TABLE 5·2 WAVELENGTHS OF MAXIMUM ABSORPTION FOR CORNING 5120 GLASSES AT 3.0 MM. AS OBTAINED AT THE NATIONAL BUREAU OF STANDARDS FOR THE SLIT WIDTHS INDICATED

Wavelength of Maximum Absorption, mμ	*Approximate Spectrum Interval Transmitted by Slits, mμ*
$441._0$	10
$475._5$	10
$528._7$	10
$585._0$	10
$684._8$	10
$743._5$	10
745	20
808	20
883	20
1067	20

While a didymium glass is highly recommended for checking the wavelength calibration of recording spectrophotometers, as noted above, there are two other uses of the didymium glass which are not recommended. First, the didymium glass is not well suited for checking the photometric scale of any spectrophotometer, recording or nonrecording. Transmittances at the peaks of the absorption bands are too dependent on slit widths, and transmittances on the steep parts of the curve are too dependent on slight wavelength errors, both as illustrated by Fig. 5·8. Second, the use of a didymium glass to check the wavelength calibration of a nonrecording spectrophotometer is considered much inferior from the standpoints of time, convenience, and reliability to the direct use of line sources as described above. The National Bureau of Standards has consistently refused to accept didymium glasses for calibration for either of these two purposes.

D·PHOTOMETRY

Another of the principal parts of a spectrophotometer is the photometric system, whereby the desired ratios of energy or flux may be determined at the various wavelengths isolated by the spectrometric system. Important parts of the photometric system are the source and detector of radiant energy, and these will be considered before discussing the photometric devices themselves.

5·9 Spectral characteristics of sources and detectors. Various sources of radiant energy have proved adequate for spectrophotometry in the range from 200 to 1000 mμ. From 350 or 400 mμ to beyond 1000 mμ the incandescent-filament lamp is by far the most suitable source for most purposes, either as a standard of spectral energy distribution when measuring the distribution of other sources or as the source for spectral transmission and reflection measurements. Its advantages relate to the facts that (1) its radiant intensity under normal operating conditions is adequate for the purpose; (2) its intensity can be readily kept constant, to a high degree if necessary; (3) having a continuous spectrum, it enables measurements to be made at any wavelength over its useful range.

In Fig. 5·9 are shown the relative spectral energy distributions of an incandescent source at color temperatures of 2600 and 3000°K. Most incandescent lamps used on spectrophotometers operate within or close to this range. The data plotted are those from Planck's equation for the theoretical black body at these temperatures (*29*, *20*, *102*), but it is known that they closely represent the relative distributions of lamps at these color temperatures (*27*, *96*). Below 350 mμ, however, absorption of the ordinary glass bulb rapidly reduces the energy to zero near 300 mμ. This is apparent from the spectral transmittance curve shown in Fig. 5·9 for a piece of glass cut from a 200-watt incandescent lamp bulb.

The quartz-mercury lamp is useful not merely for wavelength calibration of spectrophotometers; at certain wavelengths the radiant intensity is sufficiently great that it may also be used as the source for photometric measurements. The advantages of such use are that the results cannot be affected by wavelength or slit-width errors, as is possible with an incandescent lamp as source.

The mercury lines that have been found useful for such purpose at the National Bureau of Standards are (1) visually, on the König-Martens spectrophotometer, those at 404.7, 435.8, 546.1, and 578.0 (576.9 + 579.0) mμ; and (2) photoelectrically, on the Beckman spectrophotometer, those at 365.0, 334.2, 302.2, and 253.6 mμ in addition to those listed above in the visible spectrum. The relative intensities among various of these lines vary considerably from one type of lamp to another. One such set of intensities is shown in Fig. 5·10 (*78*). In this figure the intensities below 400 mμ are taken without change from Table XVII of reference (*78*). Above 400 mμ, however, they have been adjusted to fit the individual lines. The data of Fig. 5·10 are for a "hot" quartz lamp. The relative intensities for a low-pressure, "cold-cathode" mercury lamp are much different, the intensity of the line at 253.6 mμ exceeding all the others.

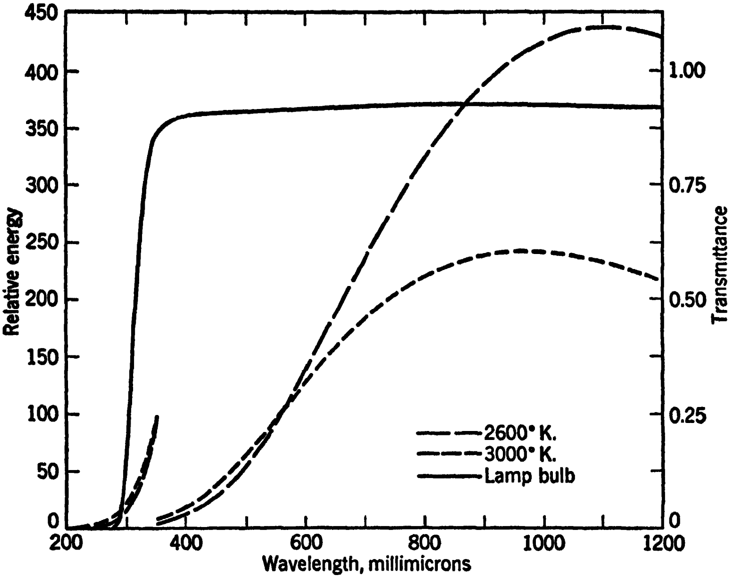

FIG. 5·9. Spectral energy distribution curves of black body at 2600° and 3000°K., adjusted to 100 at 560 and 350 mμ. These curves are closely like those from the incandescent lamp filaments used on spectrophotometers, except that in the ultraviolet below 350 mμ they are notably reduced by the transmittance of an ordinary glass bulb. The spectral transmittance curve (continuous line) of such a bulb is shown.

The most commonly used source of radiant energy for spectrophotometry in the ultraviolet spectral region is probably now the hydrogen arc. Under certain operating conditions of pressure and current a nearly continuous spectrum can be obtained, of adequate intensity for use over the range from 200 to above 400 mμ. Hydrogen arcs are suitable for both photoelectric and photographic spectrophotometers.

Other sources widely used in photographic spectrophotometry, but too unsteady for the usual type of photoelectric spectrophotometer, are (1) the underwater high-voltage spark discharge and (2) various arcs or sparks where the electrodes have been especially selected to yield a great number of closely spaced spectral lines. The underwater spark spectrum has long been used at the National Bureau of Standards, and its appearance is shown later in Fig. 5·16. The spectrum is perfectly continuous without superposed lines and covers the range from 500 to 220 mμ and perhaps beyond. Examples of arc or spark spectra in air, as used widely by various investigators in ultraviolet absorption spectra, may be found in various publications (*8*, *109*).

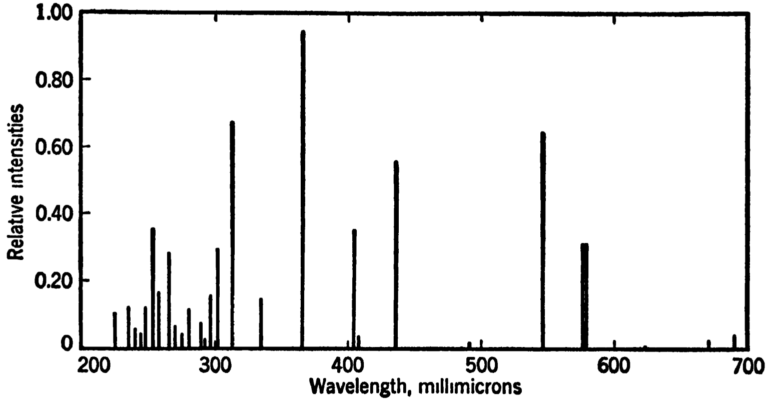

Fig. 5·10. Relative intensities of the various spectral "lines" in a quartz-mercury arc. Such a source may be used for the wavelength calibration of spectrophotometers, and the stronger lines may be used also for photometric measurements. The relative intensities differ with the operating conditions and types of arcs. Those shown are for a "hot" quartz arc. For a low-pressure "cold-cathode" arc the major portion of the intensity is concentrated in the 253.6-mμ line. Wavelengths are given in Table 5·1. (The intensities shown are based on data from Luckiesh, *Applications of Germicidal, Erythemal, and Infrared Energy*, copyright by D. Van Nostrand Company, 1946, and are reproduced by permission of the author and the publisher.)

The detectors commonly used with spectrophotometric equipment over some part of the range from 200 to 1000 mμ are (1) the eye, (2) the photographic plate, (3) the photoelectric cell (the vacuum or gas-filled electron-emission type or the barrier-layer type), and (4) the thermopile or other form of radiometric device. Only the first three types of detectors will be considered here, since the thermopile and other radiometers are fully treated in Chapter 8.

The relative sensitivity (luminosity) curve of the human eye, that is, the photometric response of the eye to an equal-energy spectrum, has been determined by various investigators. It varies considerably from one individual to another and is widely different at low brightnesses (scotopic vision) (*111*) and at high brightnesses (photopic vision) (*40*). In Fig. 5·11, curves *A* and *B*, are shown the average photopic and scotopic luminosity curves plotted to unit maxima. The shift of the curve towards the shorter wavelengths with decreasing brightness is known as the Purkinje effect. In most visual spectrophotometry the eye of the observer is probably in varying states of adaptation between the two extremes, depending on the part of the spectrum in which measurements are being made and the room brightness within his field of view.

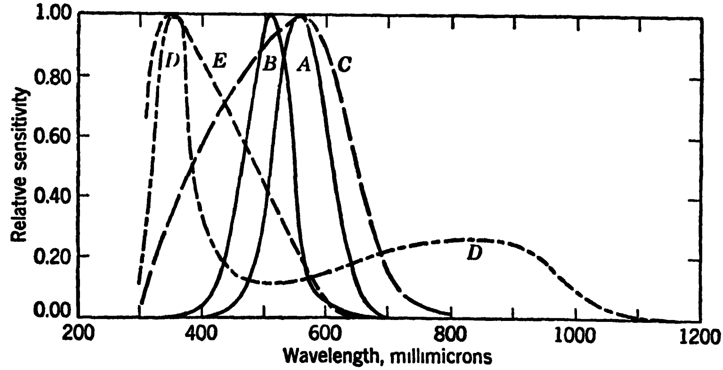

Fig. 5·11. Relative sensitivities of various detectors used in spectrophotometry. *A* and *B* are the photopic (light-adapted) and scotopic (dark-adapted) average luminosity curves of the human eye. *C* is more or less representative of barrier-layer photocells, *D* and *E* of certain "red-sensitive" and "blue-sensitive" electron-emission phototubes. The sensitivity of *E* continues adequate to at least 200 mμ. Differing greatly from these curves are the response curves of the respective detectors at the exit slits of spectrophotometers resulting from energy distribution of source, prism dispersion (if not compensated by varying slits), and instrumental transmittance.

The spectral responses of various types of photocells, including several types of sensitive surfaces, differ enormously (*26*, *66*). Even for photocells of a given type and surface the responses may vary considerably, somewhat similarly to the luminosity curves of different individuals. In Fig. 5·11 are shown spectral sensitivity curves of certain types of photoelectric cells that have been commonly used in spectrophotometers. These are plotted relative to unit maxima and give no indication of the absolute sensitivity of the cell. They indicate relative response to an equal-energy spectrum.

Curve *C* is more or less typical of the barrier-layer cells used in the Cenco-Sheard and Coleman spectrophotometers. Curve *D* represents more or less the so-called "red-sensitive" electron-emission type of phototube used in the General Electric recording spectrophotometer, the Coleman universal spectrophotometer, and the Beckman quartz spectrophotometer. Curve *E* represents the "blue-sensitive" phototube also used in the Beckman instrument.

It should be remembered that curves *C*, *D*, and *E* of Fig. 5·11 do not represent the relative responses of these photoelectric cells at the exit slits of actual instruments. The response will be affected by the spectral energy distribution of the source used, by the spectral dispersion (for prism instruments) unless compensated by varying slit widths, and by the spectral transmittance of the spectrophotometer.

Similarly the luminosity curves, *A* and *B*, of Fig. 5·11 do not represent the relative field luminances in a visual spectrophotometer, nor does the plate sensitivity curve of a photographic plate represent the relative spectral densities obtained in any photographic spectrophotometer. In both cases the response curves (luminance or density) depend on the instrumental dispersion and transmission and the energy distribution of the source, as well as on the spectral sensitivity of the detector.

The photographic plate has rarely been used for spectrophotometry above 500 mμ, this being the effective limit for unsensitized emulsions. Absorption of the gelatin prevents adequate sensitivity below about 220 mμ. If it is desired to use photographic methods below this wavelength, special plates must be employed. These may use fluorescent dyes in the emulsion or nongelatin suspensions (*8*, *26*, *54*).

5·10 Photometric principles and devices. The design of the photometric part of a spectrophotometer depends directly upon the detector or receptor used. It may be recalled that spectrophotometry consists always of measuring the ratio of two radiant energies (or radiant fluxes) at a specified frequency or wavelength, this measurement being repeated at other frequencies or wavelengths as often as desired over the spectral range of interest. With a photoelectric cell this ratio may be determined by measuring the ratio of currents evoked by the two radiant fluxes, provided a closely linear relation can be established between the irradiance on the photocell and the current evoked in the cell and indicated in the galvanometer or other measuring device. In such cases the photometric system is mostly electrical.

A similar direct measurement of the spectrophotometric ratio by the photographic plate is difficult and by the eye impossible. In nearly all photographic spectrophotometers and in all visual spectrophotometers, therefore, this ratio is measured by some auxiliary optical system, often incorporated in the instrument, the setting being indicated by equality of luminance or of photographic density in a two-part photometric field.

a. Visual photometry. Although the eye can estimate only roughly the absolute magnitudes of photometric quantities, it can determine with considerable precision when the two properly juxtaposed parts of a photometric field are equally bright, provided, of course, that the two parts of the field are of the same chromaticity, as is usually the case in visual spectrophotometry.

In a visual spectrophotometer, therefore, the photometric part of the instrument includes (1) means for securing a uniformly bright two-part photometric field, and (2) means for varying the luminance of one or both parts of this field in a continuous, easily adjustable, and known manner, so that when the eye indicates equality of match the desired spectrophotometric ratio can be determined.

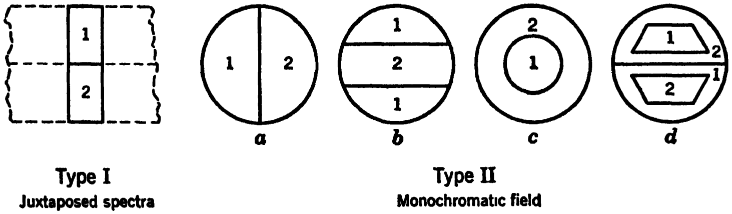

FIG. 5·12. Various types of photometric fields used on visual spectrophotometers. The eye can equate two properly juxtaposed brightnesses with considerable precision. [Reproduced by permission from *J. Optical Soc. Am.*, **10**, 169 (1925). See ref. 36.]

Various types of photometric field have been used; these are shown in Fig. 5·12. In Type I of this figure the two parts of the field consist of two juxtaposed spectra formed at the exit slit of the instrument and viewed by a magnifying eyepiece; the other fields illustrated in Fig. 5·12, type II, are formed or projected in or between the telescope and collimator lenses and are viewed by the eye placed (usually) close to the exit slit. The trapezoids of type II*d* are usually about 8 per cent darker than their surroundings (being produced by reflection from clear glass plates), and equality of contrast rather than equality of brightness is the criterion for photometric match.

Various methods have been used to vary the luminance in one or both parts of the field. (1) When two collimators are used, the slit width of one may be varied and the luminance is taken as proportional to the width. The method conserves light, but its accuracy is limited. (2) Rapidly rotating sectors have been used, having variable aperture while rotating. (3) Polarization methods have often been used, probably more than other means. An instrument using this method of luminance variation is illustrated in Section 5·11.

Still other methods are possible and have occasionally been employed. However, the fundamental inverse-square relation has been little used in visual spectrophotometry because of the resulting low brightnesses. For further information regarding various phases of visual photometry or spectrophotometry, reference may be made to Section 5·11 below or to some of the publications already listed (*36*, *38*, *81*).

When properly used, a visual spectrophotometer can give results of high accuracy. Under the best conditions the uncertainty of the mean of several settings is some fraction of 1 per cent of the value. However, the necessity of making repeated settings to obtain adequate reliability makes the method tedious and time-consuming. As an independent method in measuring standards of spectral transmittance and for certain other special problems, the König-Martens visual spectrophotometer at the National Bureau of Standards is still of great utility, but for most transmission measurements and for practically all reflection measurements the instrument and method have been superseded by more rapid photoelectric spectrophotometers.

b. Photographic photometry. When a photographic plate is exposed to radiant energy and developed, the relation between the photographic density of the developed plate and the exposure to which the plate is subjected is as shown in Fig. 5·13 (*26*). *Density* and *exposure* are terms widely used in photographic photometry, the former being defined usually as the negative logarithm of the transmittancy (ratio of luminous flux transmitted by the silver deposit to that incident upon it) of the developed photographic material, the latter representing the logarithm of the product of illuminance (light units) and the time of exposure.

The relation shown in Fig. 5·13 is the basis for much photographic photometry, a subject discussed in detail in Chapter 6. In certain photographic spectrophotometers the design is such as to eliminate dependence upon this relation, as is illustrated in Section 5·12.

c. Photoelectric photometry. As already noted, a photoelectric cell, with appropriate meter, contains within itself the possibility of direct spectrophotometric measurement, something wholly untrue of the eye and practically unfeasible with the photographic plate. If there be adequate sensitivity, the question of primary importance in direct photoelectric photometry is whether there is a linear relation between the irradiance on the photoelectric cell and the resulting indication on the meter or other device by which the photoelectric current is indicated. Lacking such linearity, there must be adequate calibration or resulting error.

For a long time such linearity for the electron-emission type of cell, now usually called a phototube, appeared to be the exception rather than the rule (*66*). For many modern phototubes, however, particuarly the vacuum type, and at irradiances of the order of those used in spectrophotometry, the linear relation is closely if not exactly followed. Some of the photoelectric spectrophotometers now on the market are based on the linearity of the irradiance-current relation. In another type of spectrophotometer this linear relation is of no importance; the phototube is merely a balancing indicator, and the photometric system is based on polarization principles similar to those in certain visual spectrophotometers.

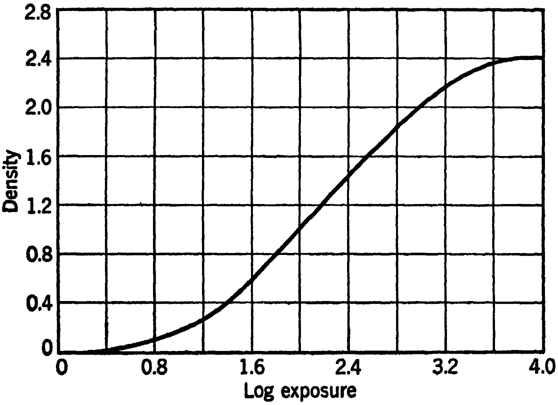

Fig. 5·13. Typical density-exposure curve of photographic emulsions. Photographic spectrophotometers are usually designed in such a way as not to be dependent on this and certain other characteristics of photographic materials. (This curve is derived from Fig. 164 of Forsythe, *Measurement of Radiant Energy*, copyright by McGraw-Hill Book Company, 1937, and is reproduced by permission of the publisher.)

The barrier-layer photocell, not requiring external voltage, has also been used in spectrophotometry. The question of linearity is likewise of importance with this photocell, and here as always the reliability of the photometric scale should be checked in one way or another if results of the highest accuracy are desired.

d. Sample holders. Four of the five principal constituents of every spectrophotometer, namely the source, the spectrometer, the photometer, and the detector, have been considered above. There remains only the sample container and the holder, carrier, or other device by which the sample may be moved in or out of the beam or from one beam to another, or interchanged with the standard or comparison sample, or merely held in place. Space is lacking to illustrate the numerous devices that have been designed for this purpose, these designs varying in turn with the type of measurement to be made, whether it be the transmittance of a glass, the transmittancy of a solution, or the directional reflectance of a textile, powder, or other type of material.

Glass or quartz cells of varying types for holding solutions and solvents are described in detail in many of the publications listed (*8, 47, 54, 81, 109*). Several important considerations relate to the use of such cells. The end plates employed in the solution and solvent cells should be identical in every respect; the internal dimensions should be accurately known; and all parts of the cells with which the solution and solvent are in contact should be capable of being thoroughly cleaned.

Another important factor in both transmission and reflection measurements involves the stability of the sample holder or carrier, and the precise reproducibility of positioning when the sample is replaced by the standard or when sample and standard are interchanged. Closely related to this in reflection measurements is the problem of the positioning of a sample such as a textile which is likely to "bulge" so that it is not in the same plane as the standard or comparison sample. Putting both samples under a cover glass may remedy this but may introduce multiple reflection errors (*71*).

E·TYPICAL INSTRUMENTS AND METHODS

5·11 Visual. It is believed that the only visual spectrophotometer commercially available at present in this country is that manufactured and sold by the Gaertner Scientific Corporation (*31*). Since this is somewhat similar to the visual spectrophotometers sold by Adam Hilger, Ltd. (*109*), and formerly sold by the Bausch and Lomb Optical Company (*3*), and also is similar in principle to the König-Martens spectrophotometer (*81*) in use at the National Bureau of Standards, it has been chosen for illustration. Where the other instruments have special points of difference, they will be noted.

The photometric part of the Gaertner spectrophotometer is outlined in Fig. 5·14, which is based on advertising circulars of the company. In this instrument, as shown, and in the Hilger and Bausch and Lomb instruments, the photometer is a separate unit from the spectrometer, whereas in the König-Martens spectrophotometer the two parts are incorporated into one instrument.

The source of radiant energy shown in the illustration is one designed for both transmission and reflection measurements. (A source designed solely for transmission measurements can be obtained if desired.) It is a white-lined sphere, 10 inches in diameter, in which sixteen incandescent 6-volt lamps are mounted around the vertical equator. For reflection measurements the two samples being compared, *A* and *B*, are held against openings in the sphere as shown in the upper part of Fig. 5·14. Specially designed diffusing screens near each lamp shield the sample and standard from direct irradiation without casting shadows. Multiple reflections from the walls of the sphere then combine to produce diffuse irradiation. This irradiation fails to be perfectly diffuse principally because of the sphere opening through which the reflected energy is taken to the instrument. Collimating lenses are placed at *L*. The type of directional reflectance measured is designated as $R_{d,0}$ (Section 5·6).

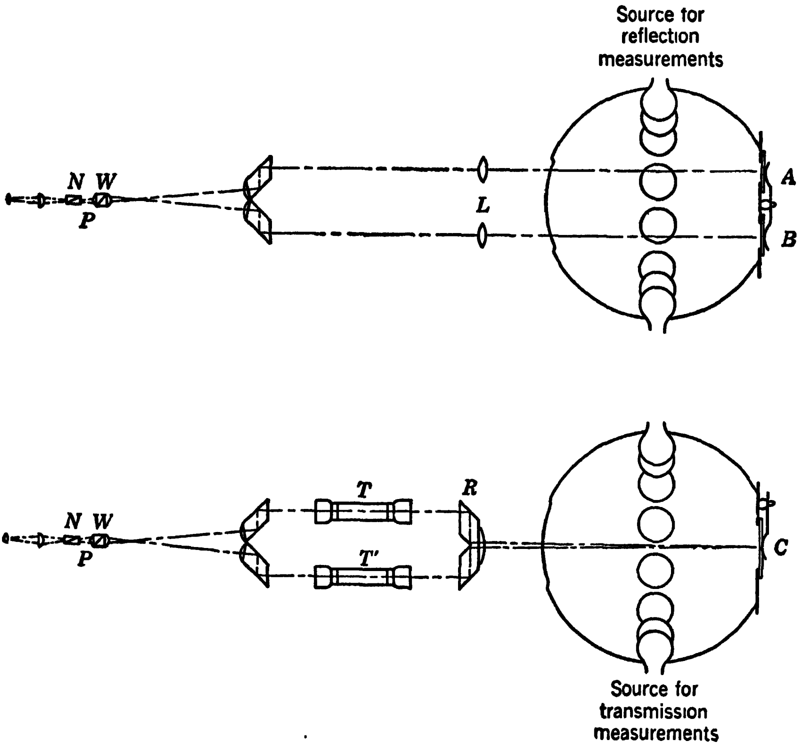
Source for reflection measurements
N W
P
L
A
B
T
R
N W
P
T'
C
Source for transmission measurements

FIG. 5·14. Optics of the Gaertner visual spectrophotometer. The spectrometer is not shown. With this equipment the samples are irradiated before the radiant energy is dispersed in the spectrometer. [Based on illustrations in Gaertner bulletins (*31*).]

For transmittancy measurements a single diffusing plate, *C*, is used, as shown in the lower part of Fig. 5·14. The radiant energy reflected from this plate is split into two beams by the pair of rhombs, *R*, and led through the solution and solvent cells, *T* and *T′*, into the photometer.

For both reflection and transmission measurements the two beams are passed by suitable optical means through the photometer and the spectrometer, and the eye placed at the ocular slit of the spectrometer sees a photometric field of type II*a*, Fig. 5·12, with dividing line horizontal.

The photometer, *P*, is the polarization type known as the Martens photometer (*79*), although the internal optics of the German and American designs are not identical. The two beams entering the instrument are led to form the two halves of the photometric field, the dividing line of which is the edge of a biprism. The light in the two halves of the field is polarized in mutually perpendicular planes by means of a wollaston prism, *W*, the planes of polarization being respectively parallel to and at right angles to the dividing line of the field (the edge of the biprism). A nicol prism, *N*, is mounted at the left of the wollaston prism, and by turning this nicol the two halves of the field may be precisely matched in brightness.

If the transmittancy of a solution is being measured, let α_0 be the angle of match with both beams blank (or containing identical solvent cells) and α_1 be the angle of match when the respective cells containing solution and solvent are placed in the beams. Then

$$T_s = \frac{\tan^2 \alpha_1}{\tan^2 \alpha_0} \tag{5·13}$$

The derivation of this formula and many other facts of interest in this connection may be found in the papers by Martens (*79*) and McNicholas (*81*). If the solution and solvent are interchanged, a similar relation is obtained. That is,

$$T_s = \frac{\cot^2 \alpha_2}{\cot^2 \alpha_0} \tag{5·14}$$

By combining these two relations it follows that

$$T_s^2 = \frac{\tan^2 \alpha_1 \cot^2 \alpha_2}{\tan^2 \alpha_0 \cot^2 \alpha_0} \tag{5·15}$$

or

$$T_s = \tan \alpha_1 \cot \alpha_2 \tag{5·16}$$

The $\tan^2$ relation is useful if one wishes to put direct-reading scales on the photometer, and this has been done by both Gaertner and

Bausch and Lomb. In this case it is essential (unless correction be made) that α_0 be exactly 45° (so that $\tan^2 \alpha_0 = 1.000$).

It may easily be overlooked in this connection that a substitution or interchange method should always be used in spectrophotometry. In a substitution method the test sample and its reference standard should be compared in the same beam by way of a third or comparison sample in the second beam. This is true in both transmission and reflection measurements. It is often convenient to have the comparison sample identical with the reference standard, but except in direct-reading instruments this is not essential.

On the König-Martens spectrophotometer at the National Bureau of Standards the interchange method is used, and T_s is computed from the tan × cot relation. This is equivalent to a substitution method, but it does not matter what the value of α_0 is, and no measurement is made of it. A reference standard is necessary, but no comparison sample.

On the Bausch and Lomb spectrophotometer means were provided for passing the beam through solution and solvent cells in which the thickness of absorbing layer could readily be varied without removing the cells.

On the Hilger Industrial and Research Spectrophotometer a similar $\tan^4 \alpha$ relation is used (*21, 109*), resulting in a more open scale at high absorbancies.

Auxiliary equipment designed and constructed at the National Bureau of Standards (*81*) for use with the König-Martens spectrophotometer includes the following features:

1. Hemispherical irradiation of the sample and standard for reflection measurements, by means of which the irradiation is in effect almost perfectly diffused. The type of directional reflectance measured is thus in effect $R_{D,0}$, which is equal to R_0 by the reciprocal relation.

2. Installation of mercury and helium sources which can be readily interchanged with the incandescent source and are valuable for use in transmission measurements as well as in wavelength calibration.

3. The installation of accurately calibrated rotating sectors by means of which (1) the photometric scale can be readily checked over the range from $T = 0.80$ to $T = 0.00883$, and (2) the transmission scale can be greatly extended at low transmissions. This extension is effected by placing the sector ($T = 0.09945$ or 0.00883) in the blank beam and measuring the transmittance of the sample relative to that of the sector.

Many other types of visual spectrophotometers have been designed and used during the past 100 years. These are described in other publications (*8, 26, 36, 38, 109*) or in papers to which these publications refer.

5·12 Photographic.* Major contributions to the science of photographic spectrophotometry have been made by Adam Hilger, Ltd. Their rotating sector photometer, introduced about 1910 for use with suitable source and spectrograph, was apparently the first commercially available equipment for reliable ultraviolet spectrophotometry, and such equipment was shortly thereafter in use in the United States (*62*).

The National Bureau of Standards equipment is described in *Natl. Bur. Standards Sci. Paper* 440 (*47*), the optics of the apparatus being outlined in Fig. 5·15. Radiant energy from the source, E, passes through cells T and T', containing solution and solvent placed in a constant-temperature inclosure. The respective beams are deviated and focused by the wedge lenses at L, then further redirected by the biprism, B, in front of the spectrograph slit; finally the two beams are made to form a pair of juxtaposed spectra on the photographic plate at P, the dividing line between the two spectra being the spectral image of the horizontal edge of the biprism, B. All optical parts are of crystalline quartz. Rotating sectors are placed at S and S'. The sector in the solution beam is kept at maximum opening, designated as 1.000 on the transmittancy scale; the sector in the solvent beam may be set at any transmittancy value between 1.000 and 0.000, this value being different for each exposure.

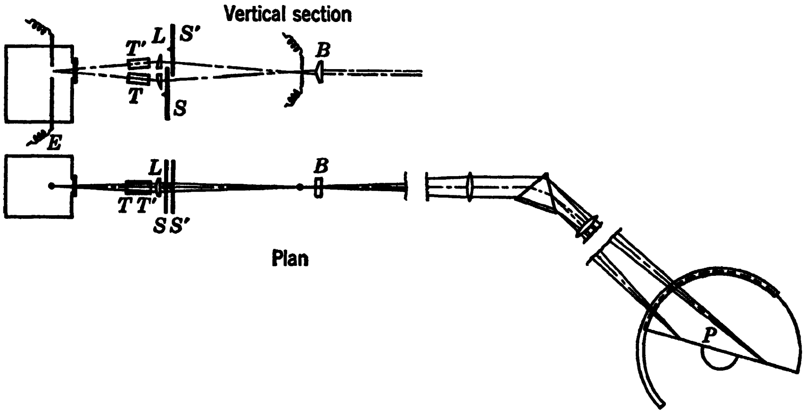

FIG. 5·15. Optics of the Hilger sector photometer, photographic spectrophotometer, as installed at the National Bureau of Standards. With this equipment the samples are irradiated before the radiant energy is dispersed in the spectrograph.

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* Chapter 6 is devoted to photographic methods. Since that chapter represents a European viewpoint, this brief section has been retained here to give an indication of the view on this technique at the National Bureau of Standards. EDITOR.

There results, when the plate is developed, a series of exposures as shown in Fig. 5·16. By determining for each exposure the frequencies or wavelengths at which the two juxtaposed spectra are of equal density, the transmittancy or absorbancy curve may be derived. On the plate at top and bottom may be seen the aluminum spark spectra used for wavelength calibration (the lines at the shorter wavelengths do not show well in Fig. 5·16), and the comparison spectra taken with both beams blank and both sectors set at 1.000. On this plate it will also be noted that the spectra in the lower half are a reverse repetition of the spectra taken on the upper half. On the National Bureau of Standards instrument both sectors were made adjustable and scales attached. To eliminate the effect of possible slight lack of match in the two halves of the comparison or 100 per cent spectra, the solution and solvent cells are interchanged in the two beams at the middle of the plate and the reverse set of exposures made. Averaging the wavelengths obtained from the respective pairs of exposures tends to eliminate error from this cause.

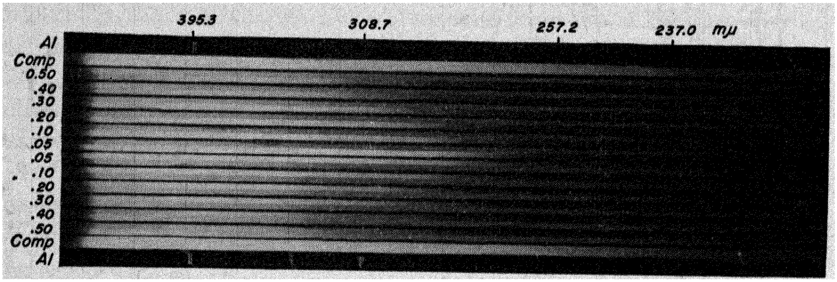

Fig. 5·16. Typical set of exposures made with equipment shown in Fig. 5·15. Note continuous spectrum extending from 400 to 220 mμ produced by high-voltage Tesla-coil spark in distilled water. For details see text.

The soundness of the principle involved in this method of photographic photometry is open to question, although at the National Bureau of Standards consistent deviations in results from those by other methods were never noted (*47*). The matter has been considered by various investigators but it is sufficient here to refer to the chapter by Jones in *Measurement of Radiant Energy* (*26*). He discusses in detail the factors involved in photographic photometry by various methods and concludes in this instance "that results are valid provided the sector speeds are sufficiently high." He further states, "It is impossible, of course, to make a general statement as to how fast sectors should be run to eliminate reciprocity-intermittency errors, but under the majority of conditions 30 interruptions per second are adequate." Since in the Hilger sector photometer both beams are interrupted by rotating sectors, it is probable that error from this cause is not appreciable even at somewhat slower speeds.

However, to eliminate this uncertainty the Hilger Company put on the market another photographic photometer designated as the "Spekker" photometer (*108*, *109*). This design eliminated the rotating sectors and changed the optics to avoid the vertical dispersion introduced by the wedge lenses and biprism (a more concentrated source can thus be used) and to produce a more nearly collimated beam through the solution and solvent. Instead of the rotating sectors, rectangular diaphragms are placed at appropriate places in the beams, and the aperture in the solvent beam can be reduced by known amounts to compensate for the absorption of the solution in the other beam. Of course, for accurate work, this requires uniformity of radiant flux per unit area across the diaphragm. A similar photometer was previously designed by Lewis (*76*), except that vanes were used in place of the diaphragms. The methods are otherwise similar to the sector-photometer method.

In this country sector photometers are made and sold by the Bausch and Lomb Optical Company (*4*) and the Gaertner Scientific Corporation (*32*) for use with certain of their spectrographic equipment. The principle in each case is the same as that of the Hilger sector photometer, but the sectors are synchronized so that variations in intensity of the source are much more certainly eliminated than with the Hilger sector equipment. Absorption tubes up to 70- or 100-mm. length can be accommodated.

Perhaps the principal defect of the above methods is the time involved in the measurements. The series of exposures usually takes a half hour or more, to which must be added the time involved in development and reading of the plate. Various schemes have been devised to speed up this process, the principal objective being to obtain the whole series of calibrated spectra at one exposure, so that instable solutions can be recorded in a very short time interval. In a design by O'Brien (*8*, *89*) a Lummer-Brodhun cube with up to twenty silvered strips alternating with the same number of clear regions is combined with a rotating logarithmic step sector, all placed just in front of the spectrograph slit and with dimensions determined by the length of the slit. Juxtaposed spectra are obtained, absorption in the solution being balanced by the reduced apertures in the step sector. In this manner the spectrogram obtained provides a direct plot of photographic density against wavelength with any value of exposure.

In another design by Twyman, a pair of Hilger echelon cells (*108*, *109*) having variable internal thicknesses are combined to give a series of juxtaposed spectra on the photographic plate. Absorption in the solution is balanced by a suitable rotating sector. Again the dimensions are determined by the length of the spectrograph slit.

5·13 Photoelectric. Numerous photoelectric spectrophotometers have been designed by various investigators, but not until the 1930's did any type become commercially available and have extensive usage. The two photoelectric spectrophotometers that have been chosen for illustration are the General Electric recording spectrophotometer and the Beckman quartz spectrophotometer. Spectral reflection as well as spectral transmission measurements can be made on both. They are both commercially available.

a. General Electric spectrophotometer. The present model of the General Electric recording spectrophotometer (*84*), originally designed by Hardy (*52, 53*), gave great impetus to colorimetric analysis and specification because (1) there was great increase in speed over previous nonrecording spectrophotometers; (2) it could make spectral reflection measurements just as readily as spectral transmission measurements; and (3) its precision and reliability were adequate for most types of measurement. The recording feature is also of great importance in many kinds of work.

A schematic diagram of the instrument is shown in Fig. 5·17, which is a reproduction (with slight changes) of Fig. 1 of Michaelson's paper (*84*). Radiant energy from the lamp forms a prismatic spectrum in the plane of the mirror at the left of the figure. A second slit here isolates a small part of the spectrum and passes it on through prism No. 2. This second dispersion spreads the stray energy into a secondary spectrum in the plane of slit No. 3. This third slit obstructs most of this stray energy but transmits most of the spectral band isolated by the second slit. Thus there passes into the photometer a spectral band of width determined by the slits used and almost wholly free of stray energy from other spectral regions. As the spectrum is traversed, the mechanical widths of the slits are continually changed by cams so as to transmit a constant amount of spectrum expressed in terms of millimicrons. The usual nominal width is 10 mμ over the range from 400 to 700 mμ, but instruments with 4-, 5-, and 8-mμ widths have been made, and the wavelength range has occasionally been extended to 750 mμ (and with wider slits to above 1000 mμ), as on the National Bureau of Standards instruments.

The radiant energy transmitted by slit No. 3 passes in turn through (1) the balancing rochon prism No. 1, (2) the wollaston prism, where it is split into two beams polarized at right angles to each other, (3) the rotating rochon prism No. 2, mounted in the hollow shaft of a synchronous motor, (4) the separating decentered lenses, (5) the transmission compartment, and (6) the sphere openings, and is finally incident upon the reflecting samples at B and C.

As rochon No. 2 rotates, the respective radiant energies incident upon B and C alternately go through successive maxima and minima, each maximum on B coinciding in time with the minimum on C. The instantaneous values of these alternating components are given in the following expressions (*52*):

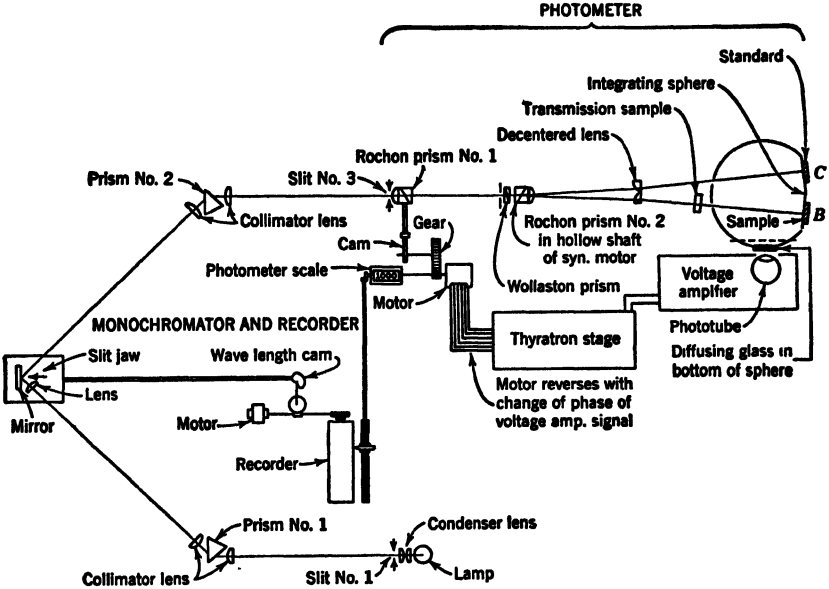

FIG. 5·17. Schematic diagram of General Electric photoelectric recording spectrophotometer. On this instrument the radiant energy is spectrally dispersed before incidence upon the samples. [Reproduced by permission from *J. Optical Soc. Am.*, **28**, 365 (1938). See Michaelson (*84*).]

$$I = I_1R_1 + I_2R_2 \qquad (5\cdot17)$$

$$= A^2(R_1 \sin^2 \alpha \sin^2 \omega t + R_2 \cos^2 \alpha \cos^2 \omega t) \qquad (5\cdot18)$$

Let $A^2 = 2$, for convenience; then

$$I = (R_1 \sin^2 \alpha + R_2 \cos^2 \alpha) - (R_1 \sin^2 \alpha - R_2 \cos^2 \alpha) \cos^2 \omega t \qquad (5\cdot19)$$

Since the photoelectric current is a linear function of the light intensity, at least over a small range, this equation, when multiplied by a suitable proportionality constant, represents the instantaneous value of the cell current. It will be seen that the cell current consists of a steady component proportional to $R_1 \sin^2 \alpha + R_2 \cos^2 \alpha$ and an alternating component of frequency $2f$ and amplitude proportional to $R_1 \sin^2 \alpha - R_2 \cos^2 \alpha$. Since the prism (rochon prism No. 2) rotates at 1800 r.p.m. the frequency of the alternating component is 60 cycles per second (*52*). ($2f = \omega/\pi$).

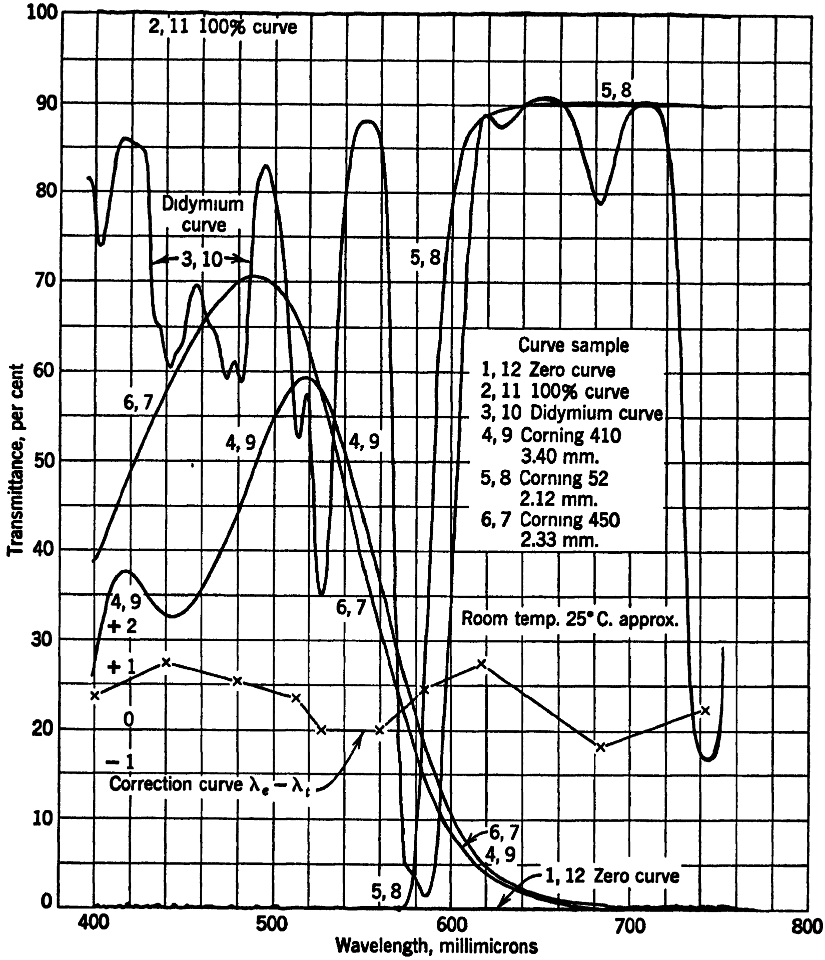

Fig. 5·18. Typical spectral transmittance curves recorded on a General Electric spectrophotometer at the National Bureau of Standards. Zero, 100%, and didymium calibration curves are also shown. The didymium glass transmittance curve is used for wavelength calibration of the sheet, and the wavelength correction curve is shown plotted. Nominal slit widths used are equivalent to 4 mμ of spectrum.

A photoelectric cell of the cesium oxide type is placed just beneath a ground-glass window placed in the bottom of the sphere. This phototube is connected, through an a-c voltage amplifier and a "thyratron stage," so that any alternating current from the photocell will in effect drive the balance motor, the direction of turn of the motor depending on the phase of the current. This phase will, in turn, depend on which of the two radiant energies reflected from *B* and *C* is the greater.

The motor is geared through a cam arrangement so that, as the armature turns, it turns rochon prism No. 1 and always in the direction that will balance the two radiant energies reflected from *B* and *C*. When these two reflected radiant energies are equal, there is no torque on the motor and the motor and rochon are at rest.

In operation another motor (at the left) runs steadily; through cams it changes the wavelength of the radiant energy passing slit No. 3 by moving the second slit through the spectrum, and at the same time it turns the wavelength drum on which the recording paper is placed. The final connection is made by translating through levers and cams the amount that rochon No. 1 turns, so as to operate a recording pen that moves up and down on the paper. This pen moves linearly with the transmittance or reflectance of the sample, and thus the respective curve is steadily traced through the spectrum. In addition to the normal cam, cams giving "×5," "log," and "log log" curves can be used.

Typical sheets of such recordings as regularly run at the National Bureau of Standards are shown in Figs. 5·18 and 5·19. Figure 5·18 is the record of three transmittance curves run for certain glasses as indicated. The slits for this recording were equivalent to approximately 4 mμ of spectrum. In this figure will be noted first the three calibration curves. (1) The "zero curve" is run with the sample beam blocked off. (2) The "100 per cent" curve is run with no sample in either beam. (3) The "didymium curve" is run with a calibrated didymium glass in the sample beam; this curve is for wavelength calibration of the sheet as explained in Section 5·8. After the three calibration curves, the curves for the samples tested are successively run; then the whole series of sample curves and calibration curves is repeated in reverse order. This repetition insures against erratic or gradual changes in conditions during the run. Each curve takes 2½ or 5 minutes, at the choice of the operator, depending on the selectivity.

Figure 5·19 shows a similar graph sheet for directional reflectance measurements made on the other recording spectrophotometer at the National Bureau of Standards, this instrument having slits equivalent to approximately 10 mμ of spectrum. (For reflectance measurements the "transmission sample" is of course removed.) The order and pur-

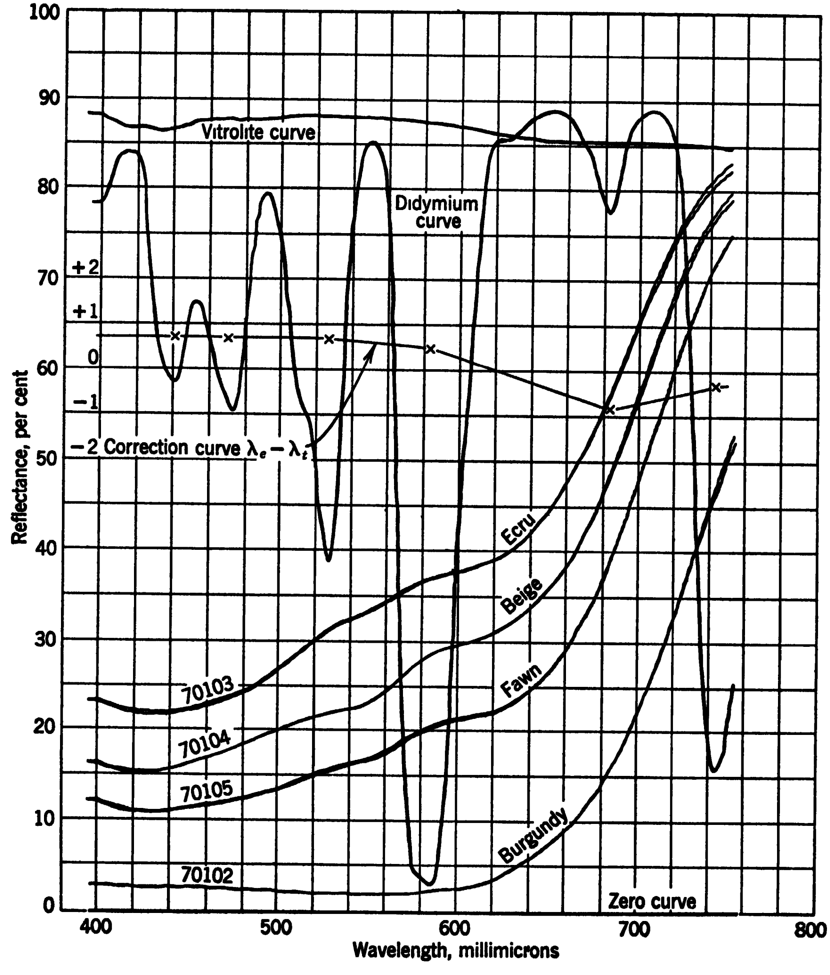

FIG. 5·19. Typical spectral directional-reflectance curves recorded on a General Electric spectrophotometer at the National Bureau of Standards. Zero, Vitrolite, and didymium calibration curves are also shown. The didymium glass serves for wavelength calibration as in Fig. 5·18, but the shapes of the correction curves on the two instruments are different. Nominal slit widths equivalent to 10 mμ of spectrum are used on this second instrument. The Vitrolite curve replaces the 100% curve and enables the reflectance data to be reduced relative to freshly prepared magnesium oxide.

pose of the respective curves are the same as in Fig. 5·19, except that a "Vitrolite curve" is run instead of the 100 per cent curve. The purpose of the Vitrolite curve is to enable all the reflectance curves to be corrected so as to be truly relative to freshly prepared MgO, the accepted standard for such work (see Section 5·24). This is done as follows.

In transmission measurements it does not matter what the reflecting materials *B* and *C* (Fig. 5·17) are, provided they will give a 100 per cent curve entirely on the network part of the sheet. (The cams are reliable for extrapolation above the network to only about 100.5 per cent.) They can be fresh or old MgO, $MgCO_3$, white glass, white paper, or other suitable material. However, it is a matter of importance that the reflectance be high, to avoid loss of sensitivity; and it is a matter of convenience that *B* and *C* be essentially identical, so that the 100 per cent curve will lie closely if not exactly on the 100 per cent coordinate line. The substitution principle is fully complied with when at any wavelength the transmittance is computed from the ratio of ordinates of the transmission sample curve to the 100 per cent curve (correcting each for the zero curve if necessary).

For spectral reflection measurements (transmission sample removed), if one is willing to prepare identical fresh MgO standard surfaces each day, the operation is similar to that for transmission measurements, except that by the substitution principle the MgO surface at *B* is *the* standard surface; the other MgO at *C*, identical or not, is merely the comparison sample. Because of the yellowing of the MgO (see Section 5·24), it is necessary that each MgO sample be newly prepared each day. If *B* is not newly prepared, it is no longer standard; if *C* is not newly prepared (and *B* is), the 100 per cent curve will rise off the network below 500 mμ. Of course, if it is known by experience that the 100 per cent curve is "on the line" and one wishes to take the chance, he can omit the 100 per cent curve and simply prepare sample *C* each morning for the day's operation.

This is risky, however, for certain kinds of work, for experience has indicated that newly prepared MgO samples may vary slightly (0.1 or 0.2 per cent) for unknown reasons. At the National Bureau of Standards it has been considered better to calibrate a working standard of directional reflectance (placed at *B*) by comparison with several different new preparations of MgO and to repeat this check occasionally to detect any possible change in the working standard.

The Vitrolite curve in Fig. 5·19 represents such a calibrated standard of directional reflectance (*74*). By its use the comparison sample *C* can be any sample of high enough reflectance to keep the recording within the network; however, in order to keep the recordings close to their true value (which has several advantages) it is well for sample *C* to be a reasonably fresh surface of MgO. At any wavelength the correct value of the directional reflectance of the sample (placed at *B*) relative to that of fresh MgO is obtained by dividing the recorded ordinate for the sample by the ratio of the recorded ordinate of the Vitrolite to the correct value for the Vitrolite relative to fresh MgO.

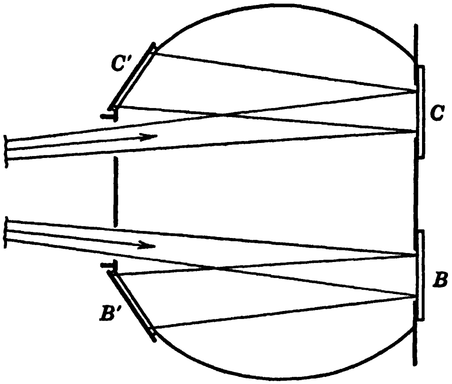

FIG. 5·20. Design of sphere used on newer models of General Electric spectrophotometer. The energy is incident at 6°, and if the surface of *B* is plane and polished the specular component of the reflected energy is received at *B'* as shown. *B'* may be a "light trap" to absorb and thus exclude this specular component from the measurements, or it may be an MgO surface to reflect and thus include this component. In the older design of sphere, shown in Fig. 5·17, the energy is incident at right angles to *B*, and part, but not all, of the specular component is reflected out through the entrance aperture.

The type of directional reflectance measured is $R_{0,d}$ with the type of sphere illustrated in Fig. 5·17; part of the specular component of the energy reflected from a plane polished surface at *B* goes back through the entrance aperture. With the newer design of sphere, shown in Fig. 5·20, the radiant energy is incident upon samples *B* and *C* at approximately 6°, and the specular component of the reflected energy is returned to the sphere apertures at *B'* and *C'* as shown. If the surface of the test sample placed at *B* is plane and polished, this specular component can be wholly excluded from or included in the measurements by either placing a completely absorbing "light trap" at *B'* or filling the aperture with a sample of fresh MgO. The respective directional reflectances may be designated as $R_{6,d}$ and $R_{6,D}$ ($= R_6$). However, two things of importance should be noted in this connection:

1. *Specular component included* ($R_{6,D}$). If the sample at B is 96 per cent diffusing and only 4 per cent specularly reflecting (approximated by a plane, polished white glass), the error involved (in $R_{6,D}$) if the reflectance of B' does not quite equal that of fresh MgO is of second order, a small fraction of the 4 per cent. However, if the sample at B is a polished mirror, so that the whole of the reflected beam is first incident upon B' before being diffused throughout the sphere, the error is of first-order importance and should be carefully considered.

2. *Specular component excluded* ($R_{6,d}$). If the surface of the sample is not plane but is mottled or irregular in flatness, as for example a piece of enameled iron may be, some of the specularly reflected energy may not be caught in the light trap at B', and the specular component is thereby not completely excluded from the measurements.

The wavelength correction curves for the graphs of Figs. 5·18 and 5·19 are shown in the graphs. The method of applying this correction is simply to read the values of transmittance or directional reflectance at $\lambda + (\lambda_e - \lambda_t)$ instead of at λ. The most effective method of reading such values is usually decided for himself by each reader; equipment facilitating the curve reading has been designed by Reimann (*98*).

Many other details relating to the General Electric or similar recording spectrophotometers may be found in papers issued from the National Bureau of Standards (*42*, *45*, *73*) and in papers published by Pineo (*92*), Stearns (*104*), and Buc and Stearns (*10*, *11*, *12*). Discussion of the error possible with fluorescent samples is given in Section 5·19.

Especially to be noted is the paper by Kienle and Stearns (*75*), in which are described the following twenty-three modifications of the General Electric type recording spectrophotometer: (1) a rotating skein holder; (2) a cam to plot the function log $[(1 - R)/2R]$, in which R is reflectance; (3) a magnified wavelength plot; (4) a magnified function of transmittancy plot; (5) a microtransmission sample holder; (6) an optical stop in the monochromator; (7) a cam to plot log log $1/T_s$ in which T_s is transmittancy; (8) a calibration filter positioning device; (9) a wavelength clutch; (10) an optical rotation sample holder; (11) a cam to plot log α, in which α is the angle of optical rotation; (12) a cam assembly permitting quick selection of cams; (13) a recorder-drum time device; (14) oblique incidence sample holders; (15) retardation plate flickering in the photometer; (16) an extended wavelength range; (17) a sliding cover for the transmission chamber; (18) variable plotting speeds; (19) a pen drive free from backlash; (20) a sample holder rejecting the specular component; (21) a horizontal sample holder; (22) retardation plate polarization-mixing of the photometer light; and (23) a black mask for absorbing specular reflectance of flat samples.

The following seven illustrative examples in which these modifications were helpful are discussed: (1) chemical analysis of reactions on fibers; (2) chemical identification in solution; (3) determination of reaction rates; (4) determination of refractive index and absorption coefficient of inks; (5) chemical analysis by transmission; (6) measurement of crystal structure; and (7) the colorimetry of chemicals.

b. Beckman spectrophotometer. The Beckman quartz photoelectric spectrophotometer has several features not found in other commercial spectrophotometers; (1) it covers the wavelength range from 210 to 1000 mμ approximately; (2) transmission measurements in the ultraviolet from 210 to 400 mμ are made as readily as in the visible spectrum; (3) directional reflectance measurements over the range from 350 to 1000 mμ are made under conditions closely equivalent to those recommended by the International Commission on Illumination in 1931 for colorimetric work.

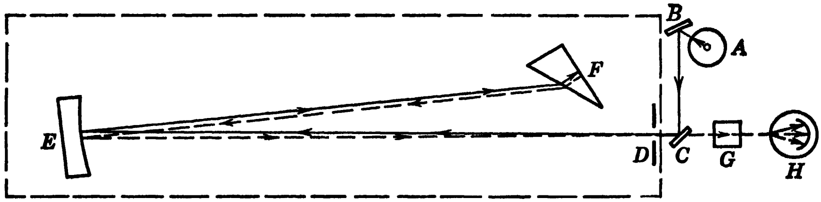

Fig. 5·21. Optics of the Beckman quartz nonrecording spectrophotometer. In this instrument the energy is spectrally dispersed before incidence on the sample. [Reproduced by permission from *J. Optical Soc. Am.*, **31**, 682 (1941). See Cary and Beckman (*14*).]

The optics of the apparatus for transmission measurements are as shown in Fig. 5·21. Radiant energy from an incandescent lamp or other source, *A*, is focused on the slit, *D*, by means of the concave mirror, *B*, and the plane mirror, *C*. The beam entering the slit is collimated by the mirror, *E*, and passes through the quartz prism to the reflecting surface, *F*. After reflection at *F* the beam returns along nearly the same path to the same slit, *D*, where it emerges slightly above the entrance beam and the mirror, *C*. After passage through the sample or sample compartment, *G*, the beam is incident on the phototube, *H*.

Two phototubes are supplied installed in the instrument, either of which may be inserted in the beam at will. One is a cesium oxide phototube (red-sensitive) for use primarily above 620 mμ. The other is a special cesium antimony phototube (blue-sensitive) for use primarily below 620 mμ. If incandescent lamp and common glass phototube are used, this range terminates at about 320 mμ. If the tube has an envelope of Corning 9740 glass, and if a hydrogen lamp with a window of the same type of glass is used as source (all supplied with the instrument when desired), the range extends to nearly 200 mμ.

The photometric scale is based on electrical rather than optical principles. The photoelectric current from the irradiated phototube produces across the load resistor a voltage drop which is balanced by a potentiometer. While this null setting is being made, any imbalance is amplified electronically and is indicated by a milliammeter on the instrument. Accuracy of the photometric scale depends, among other things, on conformity of the phototube load resistor to Ohm's law and on the linearity of the irradiance-current relationship. Since vacuum phototubes are used, the relation should hold closely. Linearity is still further assured by a frame type anode in the phototube.

Various details regarding the instrument and its operation in spectral transmission measurements may be found in a recent paper from the National Bureau of Standards (*44*) as well as in the original paper. The following points may be noted here:

1. The slits are narrower than usual on commercial spectrophotometers, normally isolating spectral regions of only 0.5 to 1.5 mμ within the range from 220 to 950 mμ. This feature of the instrument serves three useful purposes. It enables the spectral transmittance of samples having high selectivity to be determined with an accuracy unattainable with instruments having wider slits. It makes possible the precise and accurate comparison of nearly identical samples, even those having high absorption, without the use of slits unduly wide. It also makes possible the reliable determination of very low transmittances by a "step-down" procedure, using samples of intermediate transmittance.

2. No error was detected in the photometric scale on an instrument obtained at the National Bureau of Standards in 1945. These tests were made by measuring and comparing the spectral transmittances of certain glass standards on the Beckman, the König-Martens, and the Gibson spectrophotometers, and together with many other details are described in the recent publication (*44*).

3. Residual wavelength errors found on two Beckman spectrophotometers are shown later in Fig. 5·27, curves 1 and 2. A screw adjustment provides for bringing any desired wavelength into perfect alignment. The mercury wavelength 546.1 mμ has been found suitable for this purpose.

4. The quartz-mercury arc supplied with the instrument not only serves for wavelength calibration but can be used at certain wavelengths for photometric measurements (Section 7·9).

5. The hydrogen arc supplied with the instrument gives a sufficiently

continuous spectrum that transmission measurements can be made at any wavelength from 210 to 400 mμ.

6. Trouble has been experienced in the measurement of polarizing materials if the quartz plate over the entrance slit is not cut with faces perpendicular to the axis (*44*). At the National Bureau of Standards this plate has been removed.

7. The instrument has the usual amount of stray energy present for single-dispersion spectrophotometers. The purple filter supplied with the instrument should always be used with incandescent source below 400 mμ, and other stray-energy filters should be obtained and used under certain circumstances (Section 5·17).

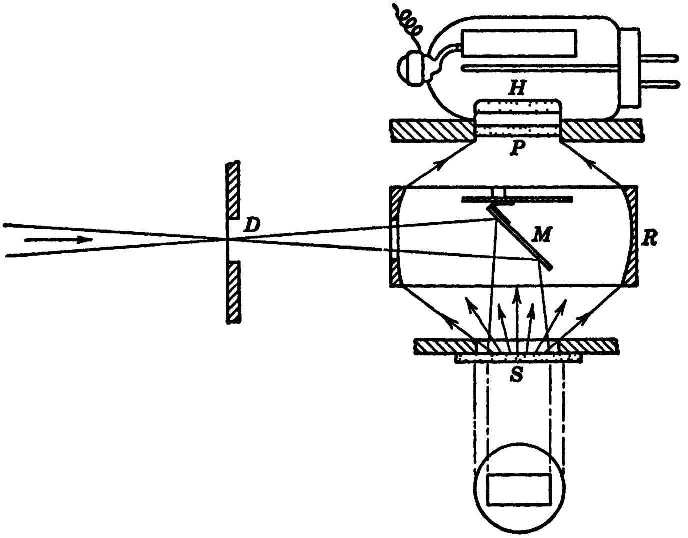

Fig. 5·22. Optics of the reflectance attachment to the Beckman quartz spectrophotometer. The incident energy from the exit slit, *D*, is directed by the mirror, *M*, so as to be incident at right angles on the sample or standard placed at *S*. The energy reflected from *S* at approximately 45° in all directions is focused by the ellipsoidal mirror ring, *R*, onto the diffusing glass, *P*, immediately under the phototube, *H*.

8. Any deviation of the beam by the sample (if wedge-shaped or lens-shaped, for example) may cause error in the measurements.

Directional reflectance measurements can readily be made on the Beckman spectrophotometer by means of special attachments obtained from the manufacturer. The optics of such measurements are shown in Fig. 5·22. The radiant energy transmitted through the exit slit, *D*, is directed downward by means of the mirror, *M*, and is incident approximately at right angles upon the sample or standard at *S*. The irradiated area is about 8 × 17 mm. A carrier enables either the sample or the reflectance standard with which it is being compared to be placed in turn in the beam.

The energy reflected from the sample or standard at approximately 45° in all directions is reflected and focused by the ellipsoidal metallic mirror ring at *R* onto the ground-glass plate at *P*. The phototube compartment is mounted on top of the attachment so that the phototube, *H*, is just above the diffusing glass.

The operation of the instrument for reflection measurements is similar in all respects to that for transmission measurements. The following points may be noted, however:

1. The slit widths necessary for normal operation are much wider than for transmission measurements. They vary from 3 to 4 mμ in the region near 530 mμ to about 8 mμ at 350 mμ, 16 mμ at 630 mμ, and 15 mμ at 750 mμ. These widths may be made to vary considerably by adjustment of the sensitivity dial, however.

2. While the axis of the reflected energy is closely at 45°, the angular aperture of the beam is approximately 20° (35° to 55°). The directional reflectance thus measured, $R_{0,45}$, is numerically equivalent to that recommended by the International Commission of Illumination, $R_{45,0}$, as noted above, except that the I.C.I. recommendation does not specify the aperture of the reflected beam.

3. In reflection measurements of fluorescent materials the values obtained are liable to the same sort of error as with the General Electric spectrophotometer (Section 5·19).

c. Other spectrophotometers. In addition to the General Electric and the Beckman spectrophotometers, there are many other photoelectric spectrophotometers of various designs that may be noted here. Some of these are commercial instruments: (1) The Cenco-Sheard spectrophotometer, 325 to 750 mμ, using concave reflection grating and barrier-layer photocell (*101*); (2) the Coleman "double monochromator" spectrophotometer, 350 to 1000 mμ, using transmission gratings instead of prisms (*17*); (3) the Coleman "universal" spectrophotometer, 300 to 800 mμ, using single grating with barrier-layer cell and designed primarily for chemical analysis in the industrial laboratory (*17*); (4) the Coleman "junior clinical" spectrophotometer, 400 to 700 mμ, designed solely for clinical analysis (*17*); (5) a Gaertner instrument, using two spectrometers and a Martens photometer, 450 to 820 mμ (*67*); and (6) the Cary recording quartz spectrophotometer, 200 to 700 mμ (*13*).

Among those designed and used by various investigators since 1930, but so far as known not put on the market [those before 1930 are listed in reference (*43*)], may be noted instruments or equipment by Preston and Cuckow (*95*), Hogness, Zscheile, and Sidwell (*59*), Schlaer (*100*), Zworykin (*114*), Harrison and Bentley (*56*), Brode and Jones (*9*), Gillod (*50*), Drabkin (*23*), Little (*77*), Studer (*106*), and Zscheile (*113*). Zscheile's instrument is of special interest because of the high precision obtainable with the Müller-Hilger double monochromator used.

Some of these papers treat the subject of photoelectric spectrophotometry in considerable detail, describing methods of wavelength calibration, slit-width and stray-energy errors, absorption cells, and other matters of interest and importance. Examination of them should be of great benefit to those who wish to study the numerous possibilities in the design of such instruments and the reliability of data obtained by their means.

5·14 Filter photometers.* For certain kinds of work it has been found adequate to isolate desired spectral regions by means of optical filters, each of which will transmit a more or less narrow region of the spectrum. Since the selection of spectral regions is dependent upon the filters at hand, the method has sometimes been referred to as abridged spectrophotometry (*110*). Instruments incorporating such means of spectrum variation are known as filter photometers, chemical photometers, or chemical colorimeters, and by various trade names. They are discussed in Chapter 4. The present section deals primarily with the filters available for such instruments.

In general, of course, what is desired is a filter that will transmit freely over a narrow range of wavelengths and absorb or reflect completely at all other wavelengths at which (1) the source emits radiant energy in important amounts and (2) the detector gives appreciable response. If either (1) or (2) is negligible over a given spectral range, the spectral transmittance of the filter is unimportant over the same range.

The most suitable filter or series of filters, for use in any particular instrument or type of work, depends, therefore, not only on the spectral range of direct interest but also on the spectral characteristics of the source, detector, and filter over a much wider range. Filters that might be adequate for use with a mercury arc might be wholly unsuitable with an incandescent source. Filters that might be satisfactory for photographic or visual use might give highly inaccurate results if employed with a phototube or thermopile as detector.

Several types of filters are available for general use. These are (1) glasses, usually two or more in combination, (2) two or more dyes in solution or incorporated into gelatin or other medium, (3) interference filters, and (4) miscellaneous types.

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* Chapter 4 deals more in detail with this subject.

General information regarding the spectral transmission characteristics of such filters, or of the individual components or selectively absorbing constituents of such filters, may be found in (1) various publications from the National Bureau of Standards (*15*, *37*, *39*, *48*), in advertising circulars of the Corning Glass Works (*18*), the Eastman Kodak Company (the Wratten dyed gelatin filters) (*24*), and the Farrand Optical Company and Baird Associates (the interference filters) (*25*, *2*), and in various other publications (*61*, *69*, *91*). Special sets of filters are also often supplied with the various instruments on the market, and information regarding the spectral transmittances of such filters may doubtless be obtained from the makers or sellers of the instruments.

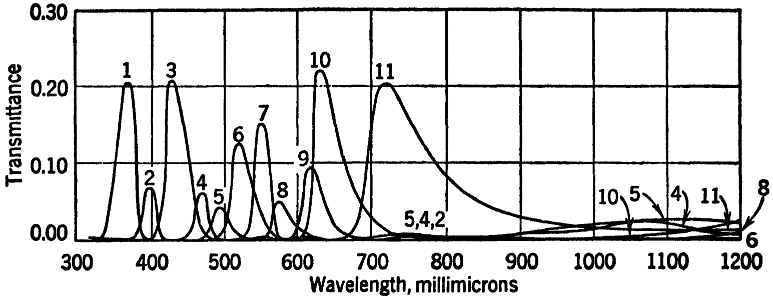

FIG. 5·23. Spectral transmittance curves of Corning glass filters (or filter combinations) which may be used for wavelength isolation in filter photometers.

In Fig. 5·23 are shown certain typical curves obtainable with glass filters sold by the Corning Glass Works; in Fig. 5·24, similar curves of certain Wratten filters; in Fig. 5·25, curves for certain interference filters; and in Fig. 5·26, curves for miscellaneous filters. All the data for the curves of these four figures were obtained at the National Bureau of Standards on samples purchased from the manufacturers, with the following exceptions: (1) the silver films of Fig. 5·26 were prepared on fused quartz by L. W. Scott of the National Bureau of Standards; (2) the data of curves 3 and 4 of Fig. 5·26 were taken from reference (*37*) and originated with Peskov, *J. Phys. Chem.*, **21**, 382 (1917), and these papers should be consulted for further details. Both the Farrand Optical Company and the Baird Associates advertise filters similar to those shown over the range from 400 to 700 mμ; in this range with these filters there is no inherent limitation on the wavelength region that may be isolated. It is understood that similar interference filters for use in the ultraviolet or infrared are in process of development.

Two factors of importance should be considered in connection with the selection and use of filters for abridged spectrophotometry, in addition to factors discussed elsewhere. One of these is the wavelength to

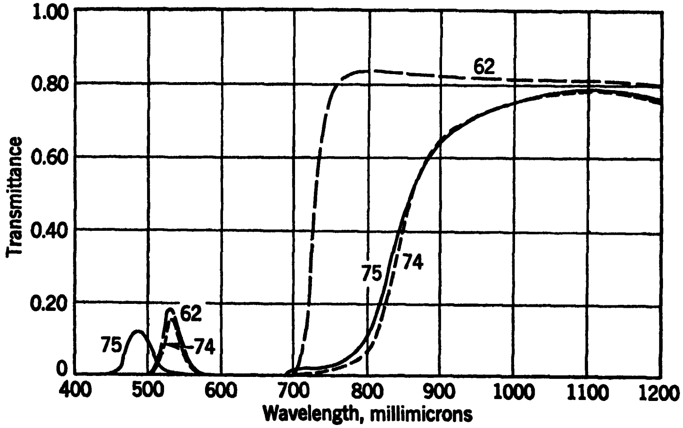

Fig. 5·24. Spectral transmittance curves of Eastman Wratten (dyed gelatin) filters which may be used for wavelength isolation in filter photometers.

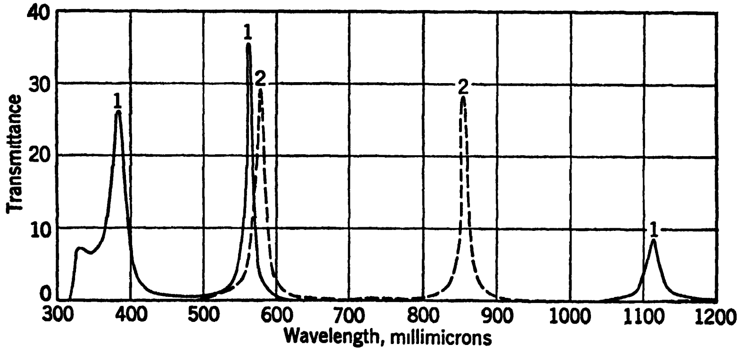

Fig. 5·25. Typical spectral transmittance curves of Baird (curve 1) and Farrand (curve 2) interference filters which may be used for wavelength isolation in filter photometers. Filters are obtainable with transmittance bands at nearly any desired wavelengths.

be assigned to the photometric value obtained by use of the filter with incandescent source; the other is an expression for the effectiveness of such filters, with respect to their transmittance at the nominal or desired wavelength and their exclusion of radiant energy at all other wavelengths. In this connection the shape of the transmittance curves shown in Figs. 5·23 to 5·26 may be compared with the shape of the slit-width "curves" of Fig. 5·6. In effect, the use of filters is more or less equivalent to the use of wide slits on a spectrophotometer, although the generality and precision of wavelength settings are missing with the filters.

If the photometric values obtained with a series of filters are to be used to plot a transmittance or reflectance curve, the correct wavelength at which to plot any particular value is of course the one for which the same value would be obtained in accurate spectrophotometry.

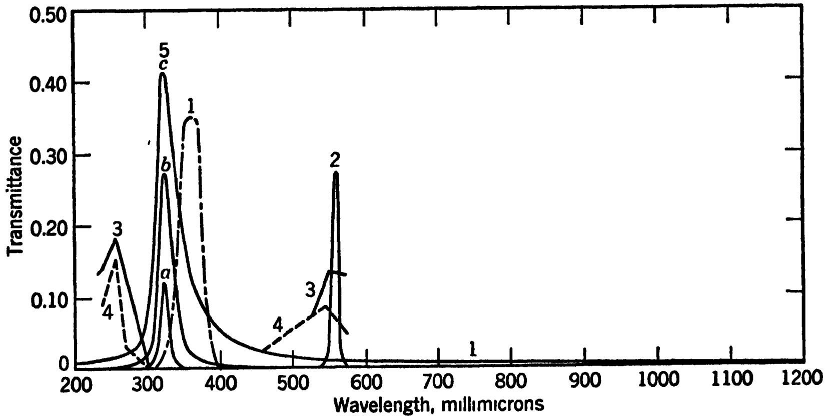

Fig. 5·26. Spectral transmittance curves of miscellaneous filters which may be used for wavelength isolation in filter photometers. Curve 1 is for Corning 5860 glass; 2, Gibson four-component glass filter; 3, quartz cell containing mixture of chlorine and bromine gas; 4, quartz cell containing chlorine gas; 5, fused quartz plates carrying silver films of the following thicknesses: (*a*) 0.0552 micron, (*b*) 0.0326 micron, (*c*) 0.0217 micron.

The photometric value obtained by use of the filter is

$$R = \frac{\int_0^\infty S_\lambda I_\lambda T_\lambda R_\lambda \, d\lambda}{\int_0^\infty S_\lambda I_\lambda T_\lambda \, d\lambda} \qquad (5\cdot 20)$$

where S_λ is the spectral response of the detector (eye, phototube, thermopile, etc.) for an "equal-energy" spectrum.
I_λ is the relative spectral energy of the illuminant.
T_λ is the spectral transmittance of the filter.
R_λ is the spectral distribution of the measured quantity.
λ is the wavelength.

Since, in general, R_λ is unknown, this relation is not useful. As a first approximation to the correct wavelength at which to plot R, it is customary to use the spectral centroid, λ_c, as defined in the following relations:

$$\lambda_c = \frac{\int_0^\infty S_\lambda I_\lambda T_\lambda \lambda \, d\lambda}{\int_0^\infty S_\lambda I_\lambda T_\lambda \, d\lambda} \tag{5·21}$$

or

$$\lambda_c = \frac{\int_0^\infty S_\lambda I_\lambda T_\lambda R_\lambda \lambda \, d\lambda}{\int_0^\infty S_\lambda I_\lambda T_\lambda R_\lambda \, d\lambda} \tag{5·22}$$

Values of λ_c by equation (5·22) need be used only for highly selective materials (R_λ varying widely over the range of T_λ), and for such cases the values of λ_c by equation (5·21) must first be obtained and R plotted as a function of λ_c yielding approximate values of R_λ. For nearly achromatic materials, such as white or near-white enamels, papers, and the like, the variation in R_λ is usually so small as to be negligible for the purpose, and values of λ_c by equation (5·21) may be used. In general, it is much more important to know the values of T_λ accurately than the values of S_λ and I_λ; and the narrower is the spectral region transmitted by the filter, that is, the more restricted is the range of T_λ, the less accurately will the values of S_λ and I_λ need to be known. The integrations are usually made by summations at every 10 mμ; but if the filter is very restrictive, 5-mμ intervals are necessary.

The effectiveness of a filter for use in abridged spectrophotometry is often given in terms of its maximum transmittance and its "half-width." By the latter term is meant the wavelength interval between the two sides of the transmittance curve, taken at a transmittance equal to half the maximum. These specifications are good as far as they go, but they ignore the radiant energy transmitted at wavelengths near the base of the transmittance curve or at some other wavelength region perhaps far removed from λ_c.

An expression for the effectiveness, E, of a filter for transmitting and isolating radiant energy at any specified wavelength, λ', has been suggested (*39*):

$$E_{\lambda'} = T_{\lambda'} - \frac{\Sigma S_\lambda I_\lambda T_\lambda | \lambda - \lambda' | \Delta\lambda}{\Sigma S_\lambda I_\lambda | \lambda - \lambda' | \Delta\lambda} \tag{5·23}$$

where the symbols have the same meanings as above, and the absolute value signs (| |) indicate that the differences are to be taken all greater than zero. Other relations for expressing the various characteristics of a filter are given in the same publication.

If, instead of an incandescent source, a mercury arc or other source is used having intense lines at suitably spaced wavelengths, the filter photometer can give photometric values at these wavelengths with all the reliability and certainty of a true spectrophotometer. The problem is simpler than with the incandescent source, in that the wavelength isolated by the filter is more definite and in many cases the exclusion of unwanted energy is less difficult. Filters specifically for use with the mercury arc in abridged spectrophotometry have been designed by various investigators (*2, 6, 18, 24, 25, 48, 103, 112*).

Numerous designs of filter photometers are on the market. Examination of such instruments will show that they combine all the essential parts of a spectrophotometer except that the series of filters replaces the more costly prism (or grating), lenses (or mirrors), and slits of the conventional instrument.

All the types of errors that are listed in Sections 5·15 to 5·21 may be present in measurements made with filter photometers. The wavelengths to be assigned to measurements with the filters were considered above. Reliability of the photometric scale is dependent on the same principles as in a true spectrophotometer; on many of the photoelectric instruments the linearity of the irradiance-current relationship must be carefully considered, and on visual instruments the soundness of the photometric device should be established in one way or another. Stray energy depends directly upon the spectral transmittance of the respective filters over the whole range of sensitivity, as already explained. Temperature effects must be carefully considered not only from the standpoint of the temperature of the sample but also because the response of a barrier-layer cell varies so markedly with temperature. The magnitudes of errors resulting from fluorescence of the sample will depend on whether the radiant energy passes first through the sample or through the filters, but will always be less important on transmission measurements than in reflection measurements. Slit-width errors can be very large because of the width of spectrum band passed by most of the filters; in some cases the error might be interpreted as a wavelength error; in either case the magnitude will depend importantly on the second derivative of the spectral transmission curve across the spectral range isolated by the particular filter being used [see equation (5·29)]. Other errors, such as deviation of the beam when the sample is inserted, the effects of multiple reflections, and electrical or mechanical instability, all must be carefully considered if the most reliable work is to be done with such instruments.

In this connection it may be noted that the types of filters desired for filter photometers in abridged spectrophotometry, such as here considered and illustrated in Figs. 5·23 to 5·26, are radically different from those necessary when the filter photometer is used for tristimulus colorimetry (see Chapter 9). In this colorimetric use, filters are desired that adequately duplicate the tristimulus functions of the human eye. Such filters are illustrated in Fig. 9·13; it will be noted that each covers a wide spectral range and would give only the crudest sort of spectrophotometric analysis.

F·ERRORS—DISCUSSION AND ELIMINATION

Numerous types of errors may be present in spectrophotometric measurements, and only the most careful calibration and operation of the instrument will render such errors negligible if the highest all-round reliability is desired. However, the importance of spectrophotometric errors varies greatly with the purpose of the measurements. One general statement is always true: the greater is the deviation between the spectral distributions of sample and standard, the more likely is the measured ratio of sample to standard to be in error.

Sometimes these errors may result from a fundamental defect in design or operation of the instrument; sometimes they result directly from carelessness or lack of training of the operator; sometimes they result from uncontrolled variables such as temperature and humidity. Whatever the cause, it is repeated here for emphasis that the importance of spectrophotometric errors depends on the purpose of the measurements. This is true for each type of error to be discussed but will not be noted further. Rather, what will be considered will be the causes of the errors and their magnitudes.

5·15 Wavelength. Two errors are to be distinguished, the error in the wavelength scale or calibration and the error thereby produced in the measured ratio. For any given wavelength error, the error induced in the ratio will depend directly on the spectral selectivity of the sample relative to the standard. This is fairly obvious; if the sample is nonselective at any wavelength or wavelengths, a wavelength error at those wavelengths induces no error in the measured ratio, but if the sample is highly selective, such as a selenium or didymium glass at certain wavelengths, an error of even 0.1 mμ has a measurable effect on the transmittance obtained. It is a simple matter for each investigator to determine the magnitude of this effect on the sample being measured.

It is believed that all commercially available spectrophotometers now have direct-reading wavelength scales. It is perhaps a matter of interest to note the residual errors found on various direct-reading wavelength scales of spectrophotometers in the Photometry and Colorimetry Section of the National Bureau of Standards. These are shown in Fig. 5·27 and give an idea of what to expect in a direct-reading instrument when put into such adjustment that the sum of these residual errors is kept to a minimum. The deviations seldom exceed ±1.0 mμ. It is to be emphasized, however, that wavelength errors as small as shown are dependent on optimum adjustment of the instrument. It is easily possible for an instrument to get badly out of adjustment, and continual check is strongly advised.

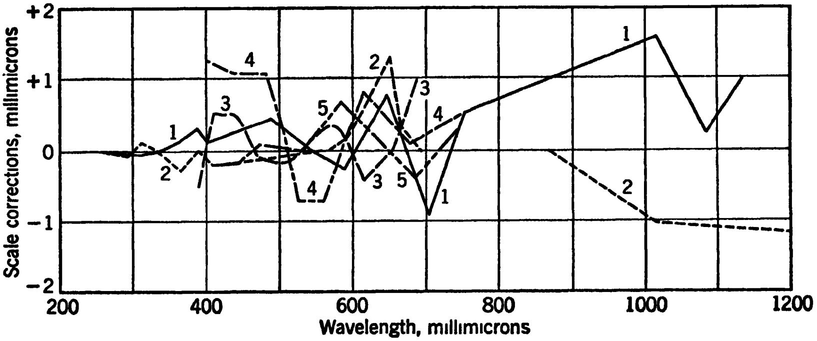

FIG. 5·27. Wavelength correction curves of various spectrophotometers in the Photometry and Colorimetry Section of the National Bureau of Standards. All the instruments are direct-reading in wavelength and are adjusted so that corrections are close to minimum. Curves 1 and 2 are for Beckman spectrophotometers; 3, for a Hilger spectrometer used in the Gibson spectrophotometer; 4 and 5, for General Electric spectrophotometers.

5·16 Photometric scale. A check of the photometric scale independent of all other sources of error is difficult or impossible to make on most spectrophotometers. Useful for this purpose would be a series of samples whose respective transmittances do not vary with wavelength, which will not displace the beam, and for which the transmittances can be independently determined with high accuracy. No such glasses or other suitable materials are available, however. Reflection samples are not good for such purpose because the directional reflectances are affected by the geometrical irradiating and receiving conditions. The use of thin metallic films does not seem advisable from the standpoints of permanence and effect of multiple reflections.

However, on visual spectrophotometers such a check is possible by means of rapidly rotating sectors. If such sectors are properly made, the fraction of the total opening relative to 360°, that is, the effective transmittance of the sector, can be measured on a circular dividing engine with uncertainties only in the fifth decimal place. Of course, the use of such rotating sectors is based on the validity of Talbot's law. Throughout the spectrum, no certain deviations from this relation are known, however. In addition to published data bearing on this (*64*, *81*) are extensive unpublished data at the National Bureau of Standards obtained over a period of many years on the König-Martens spectrophotometer. These data, obtained with sectors having transmittances over the range from 0.80 to 0.009, approximately, but principally at 0.50 and 0.10, prove both the validity of Talbot's law at the various wavelengths and the reliability of the instrument over most of the photometric scale, or else there is a remarkable balancing of errors. (The only consistent deviations are near the extinction points and are caused by stray unpolarized light, as explained in the next section.)

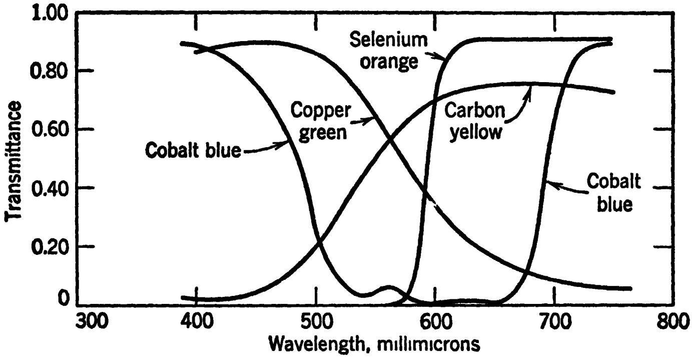

Fig. 5·28. Typical spectral transmittance curves of glass filters issued by the National Bureau of Standards for checking the photometric scale of spectrophotometers.

On photoelectric and photographic spectrophotometers similar checking of the photometric scale by rotating sectors is in general not feasible for one reason or another. Accordingly, shortly after the introduction of commercial photoelectric spectrophotometers, the National Bureau of Standards instituted the service of issuing glass standards of spectral transmittance (*44*, *49*). To date over 140 of these filters have been issued with accompanying certificates.

The spectral transmittances of these filters are approximately as shown in Fig. 5·28. A single filter thus covers a considerable part of the transmittance scale. In one sense these filters may be considered inferior to strictly neutral filters in that a deviation from the true value may be due to other causes than inaccuracy of the photometric scale. On the other hand, they are superior to the neutral filters in detecting errors resulting from stray energy, wide slits, and wavelength inaccuracies.

A similar check of the photometric scale can be made by means of the standard solutions whose spectral transmittancies are given in Section 5·23. An additional means of checking the scale is afforded by measuring the transmittancy of solutions in different thicknesses or, if Beer's law is known to be followed, at different concentrations in the same thickness. The relation, from equations (5·7) and (5·8), is

$$-\log_{10}\frac{T_s}{bc} = a_s \tag{5·24}$$

If at each wavelength tested a_s is found to be constant at the various thicknesses and/or concentrations, in all probability the photometric scale of the instrument is accurate.

5·17 Stray energy. Stray energy is often a source of important error in spectrophotometry and is produced by a variety of causes. Some of these causes are as follows:

1. The stray energy emerging from the exit slit along with the spectral energy may be a general admixture of energy of all wavelengths emitted by the source and transmitted by the spectrophotometer. It may be caused by dust, scratches, or other optical imperfections on the prism, lenses, mirrors, or other optical parts of the instrument.
2. The stray energy may be reflected from the insides of collimator tubes, the edges of diaphragms, or from other places. This may be a general admixture of all wavelengths or may be more or less localized in some other region of the spectrum than that being measured.
3. On a polarization spectrophotometer the stray energy may be manifested as unpolarized energy and may therefore not be affected by rotation of the nicol or other device by means of which the photometric value is obtained. This may produce relatively large errors near the extinction points.
4. In a grating spectrophotometer stray energy may come from other orders of the spectrum than that nominally used.
5. If the instrument or detector is not properly shielded, stray radiant energy from the room in which the spectrophotometer is used may cause error.

The spectrophotometric ratio measured is, in general, given by the following relations:

$$T_{\lambda+x} = \frac{mI_\lambda + nI_x}{mI_{0\lambda} + nI_{0x}} \tag{5·25}$$

where I and I_0 have the usual significance, λ and x refer to the spectral and the stray energy, respectively, and m and n are weighting factors dependent on the spectral sensitivity of the detector and the spectral energy of the source. Also let

$$T_\lambda = \frac{I_\lambda}{I_{0\lambda}} \tag{5·26}$$

and

$$T_x = \frac{I_x}{I_{0x}} \tag{5·27}$$

Then equation (5·25) can be rearranged to give

$$T_\lambda = T_{\lambda+x} + \frac{nI_{0x}}{mI_{0\lambda} + nI_{0x}} (T_\lambda - T_x) \tag{5·28}$$

This shows that the correct transmittance, T_λ, may be greater or less than the measured ratio, depending on whether the transmittance of the sample for the spectral energy is greater or less than its transmittance for the stray energy. The error will be zero if $T_\lambda = T_x$ or if n or I_{0x} is zero.

The proportion of nI_{0x} to $mI_{0\lambda} + nI_{0x}$ has been evaluated in certain cases (*36*, *59*). On a visual spectrophotometer it increases rapidly in the end regions of the spectrum and becomes 100 per cent in the ultraviolet and infrared. On the Beckman spectrophotometer with the present type of phototube it becomes nearly or wholly 100 per cent above 1200 mμ. The most difficult part of the ratio to evaluate is often the quantity T_x, that is, the fraction of the stray energy transmitted by the particular filter being measured.

However, a little study of the relation will usually give one an idea of whether the stray energy is of importance with any particular sample and wavelength. For example, suppose that a filter of the selenium-orange type, Fig. 5·28, is being measured at 560 mμ (where T_λ is in reality zero) with a photoelectric single-dispersion spectrophotometer containing a phototube highly sensitive to the red and infrared where the transmittance of the filter is about 0.9. The value of $nI_{0x}/(mI_{0\lambda} + nI_{0x})$ at 560 mμ might well be equal to 0.02. By inserting values in equation (5·25) or (5·28), $T_{\lambda+x}$ is found to be of the order of 0.018, an apparent transmittance of 1.8 per cent. This type of error often occurs at various wavelengths in measuring this type of glass.

Two methods are commonly used to eliminate stray energy from causes 1, 2, and 4 above.

1. The stray energy transmitted through the exit slit may be dispersed by means of a second spectrometer so that, if the first instrument

transmitted 2 per cent of stray energy at any particular wavelength, the second similar instrument would probably transmit about 0.02 × 0.02, or roughly only 0.04 per cent. This scheme has been used in certain photoelectric spectrophotometers, as noted in Section 5·13.

2. With a single-dispersion instrument the stray energy may be reduced by an appropriate filter placed at some suitable location in the optical path, which will transmit freely at the wavelengths at which it is used but absorb strongly at all other wavelengths to which the detector is highly sensitive. These filters are usually not needed in the regions of high response of the receiver but should generally be used when the spectral response becomes less than 10 or 15 per cent of the maximum. Even in regions of fairly high response they may be needed when measuring the low transmittance of a filter which has high transmittance in other spectral regions. The subject is considered in greater detail in various publications (*26*, *36*, *38*, *59*).

In the use of stray-energy filters care should be taken to avoid the introduction of multiple-reflection errors. This can usually be effected by mounting the filter so that its surfaces are not quite at right angles to the axis of the beam.

The effect of stray unpolarized light, cause 3 above, is avoided on the König-Martens visual spectrophotometer by not taking measurements too near the extinction points (0° and 90°). When low transmittances are measured, rotating sectors are placed in the blank beam, as already explained, bringing the match points back towards the center of the scale.

On the Hilger Industrial and Research Spectrophotometer (*21*, *109*) stray unpolarized light is reduced to negligible proportions by the second pair of nicols.

The effect of unpolarized energy on the General Electric recording spectrophotometer has been discussed by Pineo (*92*).

5·18 Temperature effects. The effects of temperature on the spectral transmission characteristics of many kinds of glasses and other materials have often been studied and are in many cases well known (*20*, *34*, *41*, *51*, *60*, *83*). Some typical examples are given in Figs. 5·29 and 5·30.

It can readily be seen that, with certain samples at certain wavelengths, the effect is so great that the transmittance is measurably affected by room-temperature variations or by heating of the sample in the instrument. On the same samples at other wavelengths and on other samples, the effect is immeasurably small unless studied over large temperature ranges. The effect is usually though not always small in the infrared, at least for glasses.

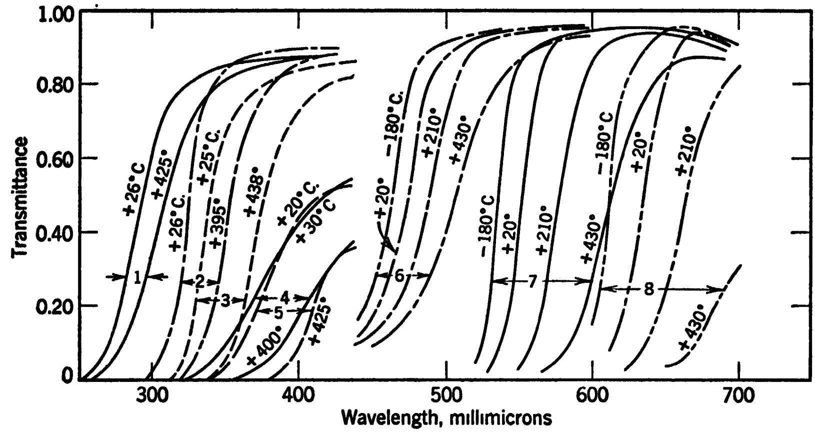

FIG. 5·29. Effect of temperature on the spectral transmittance (or internal transmittance) of certain glasses. Curves 1, 2, and 3 are for certain clear glasses; 4 and 5, for certain amber glasses; 6, for a Noviol yellow glass; and 7 and 8, for selenium orange and red glasses. [Data for curves 1, 2, 3, 4, and 5 taken from Meyer (*83*), for curves 6, 7, and 8 from Gibson (*41*).]

Some generalizations can be made. The transmittance at any wavelength usually decreases as the temperature is increased, provided the spectral transmittance is decreasing from longer to shorter wavelengths; and the greater the slope of the transmittance curve, the greater the

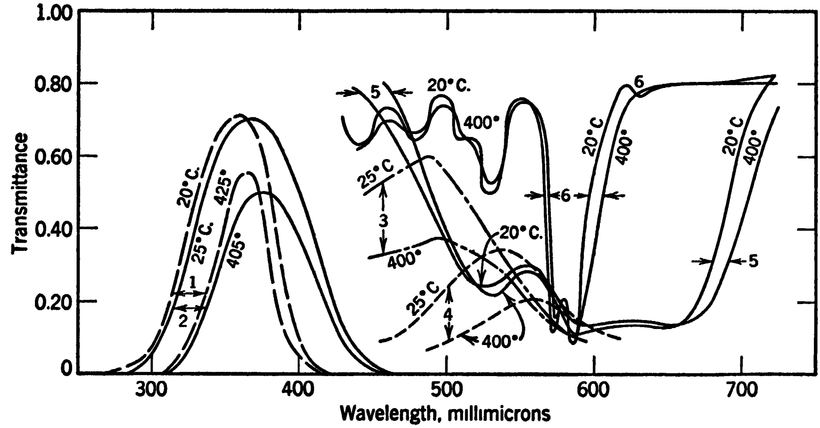

FIG. 5·30. Effect of temperature on the spectral transmittance of certain glasses. Curves 1 and 2 are for certain violet or blue-violet glasses; 3, for a copper green glass; 4, for a chrome green glass; 5, for a cobalt blue glass; and 6 for a didymium glass. [Data for curves 1 and 2 taken from Meyer (*83*), for curves 3 and 4 from Gibbs (*34*), for curves 5 and 6 from Holland and Turner (*60*).]

temperature effect usually is. If the spectral transmittance curve is not varying much with wavelength, the temperature effect is usually very small. If the spectral transmittance is decreasing from shorter to longer wavelengths, the effect is usually small and often of opposite sign.

The effects just described are elastic in that the sample always returns to a specified transmittance at a specified temperature. Corresponding changes in the spectral reflectance curves of certain samples have been observed at the National Bureau of Standards, but no quantitative study of the effect has been made.

Differing from the above are the permanent changes in the spectral reflectances of various materials (papers, dyed textiles, and the like) under strong and continued irradiation and the "solarization" of glasses when exposed to intense ultraviolet radiant flux (*16*), but such effects are not within the scope of this chapter.

5·19 Fluorescence. The accurate measurement of the spectral transmittance or reflectance of strongly fluorescent materials has not yet been satisfactorily treated from the standpoint of instrument design, especially for reflecting materials. In the various types of instruments illustrated above, it may have been noted that in some designs the samples are mounted between the source of radiant energy and the spectrophotometer. In other designs the samples are mounted between the instrument and the receptor. In the first type the full flood of undispersed radiant energy is incident upon the sample and may cause temporary or permanent changes in the sample during the measurements. In the second design the radiant energy is dispersed in the instrument and only the small spectral region isolated by the exit slit falls upon the sample, thus in general avoiding the excessive temperature or photosensitive effects of designs of the first type. For nonfluorescent samples the two designs are inherently equivalent and will give the same results under otherwise identical conditions.

With strongly fluorescent samples this may not be true, however. Both methods may produce erroneous results, but the second type of instrument is subject to much the greater error. In evaluating this error, however, one must define what is to be understood as the correct result. The effect has been most notable in instances in which it was desired to derive colorimetric specifications of strongly fluorescent reflecting materials from the spectrophotometric data (*99*). In such cases the correct result is considered to be a spectral curve or set of data consisting of (1) the true spectral directional reflectance, to which has been added (2) the spectral distribution of the fluorescent energy when excited by a source having the same spectral distribution of energy (ultraviolet, visible, infrared) as is used in the specified visual examination of the sample. This latter restriction is essential because the amount and distribution of the fluorescent energy may vary considerably with different irradiants.

If the colorimetric specification of the fluorescent sample is desired for an incandescent irradiant, a spectrophotometer of the first type (such as the Gaertner visual) can give a correct result. If it is desired for artificial daylight, a correct result can be obtained if the artificial daylight is used as source in place of the bare incandescent light. If it is desired for natural daylight, it cannot be accurately obtained unless the illumination sphere be discarded and the sample and standard be actually irradiated by natural daylight. (Natural daylight and artificial daylight may differ considerably in the ultraviolet region.)

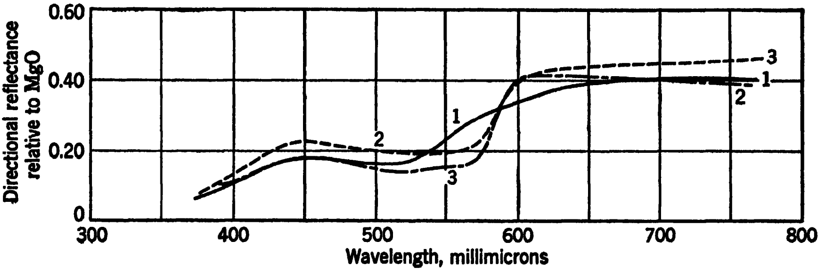

FIG. 5·31. Effect of fluorescence on the measured values of spectral directional reflectance. The sample was a pink phenolformaldehyde that fluoresced strongly to green and yellow energy but weakly or not at all to blue, violet, and ultraviolet energy. Curve 1 ($R_{0,d}$) was obtained on a photoelectric spectrophotometer in which the energy was spectrally dispersed before incidence on the sample; the high values from 500 to 580 mμ result from the reddish fluorescence excited by the green and yellow energy. Curves 2 ($R_{D,0}$) and 3 ($R_{45,0}$) were obtained with visual spectrophotometers (data extrapolated at ends) in which the sample is irradiated directly by the source, and the fluorescence excited is spectrally dispersed before measurement. Colorimetric comparison of the sample with nonfluorescent standards of nearly the same color showed that colorimetric values derived from curve 1 were much more in error than those derived from curves 2 or 3.

On spectrophotometers of the second type the fluorescent sample is irradiated by the continually changing region of the spectrum isolated by the exit slit and is therefore subject to widely varying irradiances during the measurements, probably none of which are of any particular interest in the actual use of the fluorescent sample. The magnitude of such an error is shown in Fig. 5·31, these curves relating to the sample for which the effect was first noticed (*46*).

Explanation of the effect is readily seen. The sample in Fig. 5·31 exhibited a strong reddish fluorescence when excited by energy in the green and yellow-green parts of the spectrum. The sample was run on the General Electric recording spectrophotometer, which uses a phototube that responds freely to the red and near infrared. When the sample is irradiated by energy in the green and yellow parts of the spectrum (500 to 580 mμ), the strong reddish fluorescent energy evoked falls directly (undispersed) upon the red-sensitive phototube and is added to that truly reflected at the respective wavelengths in the green or yellow. The resulting reflectance curve, No. 1 of Fig. 5·31, is thus erroneously high in the green region. The same type of error occurs on the Beckman spectrophotometer, although the choice of two phototubes for the measurement can reduce the error somewhat in some cases.

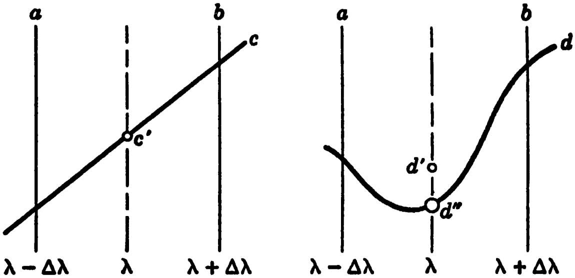

FIG. 5·32. Diagrams showing that slit-width errors depend on the curvature, not the slope, of the distribution curve across the slit. The slit width extends from a to b, $\lambda \pm \Delta\lambda$. At the left the measured and true values coincide at c'. At the right the measured value is at d', the true value at d''. See also the effect of slit width in Fig. 5·8.

The effect is much greater in reflection than in transmission measurements. In the latter the fluorescent energy is emitted in all directions, only a small fraction being incident upon the phototube, and this can be reduced by moving the sample as far as possible from the phototube or by a judicious use of filters. In reflection measurements, however, the proportion (geometrically) of the fluorescent energy accepted for measurement is the same as that of the diffusely reflected energy.

5·20 Slit width. The magnitudes of slit-width errors depend primarily upon two things: (1) the effective width of the entrance and exit slits with a continuous spectrum source, and (2) the curvature (not the slope) of the spectral distributions under consideration. The principle can readily be seen from the simple illustrations in Fig. 5·32. Let a and b be the edges of a slit of width $2\Delta\lambda$, and let λ be the nominal wavelength at which the spectrophotometer is set. Furthermore, let c and d represent two energy distributions that are being evaluated for wavelength, λ. The detector will integrate the energy transmitted by the slit over the range $2\Delta\lambda$, and the value obtained will be taken as for λ. It is apparent that for distribution c a value of c' is obtained which is the correct value. For distribution d, on the other hand, a value of d' is obtained which is different from the true value d''.

The effect of slit widths is easily shown on a recording spectrophotometer with a didymium glass. Two such curves are recorded in Fig. 5·8, one for 4-mμ slits and one for 8-mμ slits. Note that the differences are greatest where the curvatures are sharpest and that at certain places on the steep sides of the bands (where the curves cross) the differences are zero.

Mathematical expressions to evaluate slit-width errors have been derived by Runge [apparently first published in a paper by Paschen (*90*)] and by Hyde (*65*) and are discussed on pp. 182–188 (by Weniger) and pp. 349–351 of *Measurement of Radiant Energy* (*26*). Equation (80), p. 349, from that book is perhaps the most useful for spectrophotometry, namely,

$$T = \frac{\Phi_T(\Theta)}{\Phi(\Theta)} = \frac{F_T(\Theta) - K\Delta^2 F_T(\Theta) + L\Delta^4 F_T(\Theta) + \cdots}{F(\Theta) - K\Delta^2 F(\Theta) + L\Delta^4 F(\Theta) + \cdots} \qquad (5\cdot 29)$$

where T is the correct transmittance or reflectance of the sample at the wavelength under consideration.

Θ is the angle of deviation at this wavelength.

$\Phi_T(\Theta)$ and $\Phi(\Theta)$ are the relative intensities of the pure spectra as evaluated by the detector, with and without the sample in the beam.

$F_T(\Theta)$ and $F(\Theta)$ represent the relative intensities of the corresponding impure spectra resulting from the use of finite slits.

The Δ^2 and Δ^4 functions are dependent on the second and fourth derivatives of these spectral distributions.

K and L are constants, L much smaller than K.

Note the absence of functions dependent on the first derivatives and the major importance of the second derivatives in this relation, consistent with Figs. 5·8 and 5·32. Hyde's paper (*65*) should be consulted for a more complete presentation of the relations, including the definitions of the Δ^2 and Δ^4 functions and indications of how to apply the corrections.

For a given sum of entrance and exit slits, the errors or corrections are least when the two slit widths are equal. For this case,

$$K = 1/12 \quad L = 1/90$$

The obvious difficulty or bother of applying the slit-width correction, which at best is only an approximation, illustrates the desirability of keeping the slits sufficiently narrow that correction is unnecessary for the purpose at hand. It also illustrates the value of having auxiliary sources, such as the mercury arc, for use on a spectrophotometer, eliminating any possibility for slit-width error at the wavelengths available.

5·21 Miscellaneous. Various other errors are possible in spectrophotometric work, some related to particular types of measurements, some related to particular instruments.

One that is often present is a multiple-reflection error, occurring especially when the transmittance of a sample is measured relative to the blank beam. The introduction of the sample being measured in effect changes the value of I_1 in the defining relation

$$T = \frac{I_2}{I_1} \tag{5·2}$$

This can happen in various ways. The energy reflected from the sample, which should not reach the detector, may have a small part of it redirected toward the detector by another optical part. The energy reflected from a shiny slit head may have a small part of it redirected by the sample into the slit (sample between source and slit), or the energy reflected from a phototube may have a small part of it redirected by the sample back onto the phototube (sample between slit and phototube). The error can usually be discovered, if present, by measuring the transmittance of a thin piece of crown glass placed first in its normal position at right angles to the beam and then at some small angle from this position. In this second position the reflected energy is directed out of the beam without affecting the true transmittance appreciably. The remedy will then depend on circumstances but should be such that the sample being measured can be inserted at right angles to the beam without introducing the error.

In polarization instruments the extinction points should be carefully checked and adjusted if in error. In direct-reading instruments the 100 per cent point or curve should be checked (and evaluated if not precisely 100.0) to insure that the substitution method is being in effect employed.

If the transmittance sample is wedge-shaped, lens-shaped, or merely thick, its introduction into the beam may in effect change the I_1 reading. The possibility and magnitude of this error depend on the design of the instrument.

G·STANDARD DATA

Standards of spectral transmittance or transmittancy are of importance in checking the photometric scale of spectrophotometers, as already explained, and such standards are useful whether the instruments are used for spectral transmission measurements or for measurements of spectral directional reflectance or spectral energy distribution. In general, no further standards are needed for transmission measurements, since practically all measurements are made on nondiffusing materials relative to the blank beam or to a solvent cell.

Reference or "100 per cent" standards are needed, however, for spectral reflection or spectral energy measurements. While it might be possible to design an "absolute" spectroreflectometer for diffusely reflecting materials, such has not been done, and a diffusely reflecting sample or standard of known spectral directional reflectance is accordingly necessary. For spectral energy measurements a standard of known spectral distribution is likewise necessary if values for the unknown source are to be evaluated from the spectrophotometric ratio.

5·22 Spectral energy standards. The most suitable standard of spectral energy distribution through the visible spectrum is the incandescent-filament electric lamp operated at a specified color temperature. The color temperature of such a source is defined as the temperature at which the Planckian radiator or "black body" would have to be operated to give the same color as the incandescent-filament lamp (see Chapter 9). When the incandescent lamp is thus operated at a specified color temperature, the relative spectral energy distribution through the visible spectrum is given by Planck's equation to a considerable degree of accuracy. This relation is (*26*):

$$I_\lambda = \frac{AC_1\lambda^{-5}}{e^{C_2/\lambda\theta} - 1} \tag{5·30}$$

in which I_λ is the spectral radiant intensity (or some quantity proportional thereto).

e is the base of natural logs.

θ is the absolute temperature in degrees Kelvin.

A, C_1, and C_2 are constants.

Differences between the measured relative energy distribution from an incandescent-filament lamp operated at a specified color temperature and the relative energy distribution derived from Planck's equation are so slight (*27*, *96*) as to border on the uncertainties of measurement, and it has become customary therefore to accept incandescent-filament lamps as adequate standards of spectral energy distribution through the visible spectrum. Numerous publications have been issued in which these distributions have been computed and tabulated so that one does not need to derive the values for himself (*20, 29, 102*).

In Table 5·3 is shown the relative energy distribution of the Planckian radiator at 2848°K. (C_2 = 14,350 micron degrees). This energy distribution was recommended in 1931 by the International Commission on Illumination as one of three standard illuminants for colorimetry (Chapter 9) and has come to be known as I.C.I. standard illuminant *A*. It is perhaps as suitable as any other as a standard of spectral energy distribution. At lower color temperatures the radiant intensities become too low, particularly at the shorter wavelengths, to serve as a suitable standard. At higher color temperatures the lamp ages more and more

Table 5·3 Relative Energy Distribution of Planckian (Black Body) Radiator at 2848°K. (C_2 = 14,350 Micron Degrees), I.C.I. Standard Illuminant *A*

(The same distribution is given by 2842°K. (C_2 = 14,320) on the former international temperature scale, and by 2854°K. (C_2 = 14,380) on the new international temperature scale.)

Wavelength, mμ	*Relative Energy Distribution*	*Wavelength, mμ*	*Relative Energy Distribution*
380	9.79		
90	12.09		
400	14.71	600	129.04
10	17.68	10	136.34
20	21.00	20	143.62
30	24.67	30	150.83
40	28.70	40	157.98
450	33.09	650	165.03
60	37.82	60	171.96
70	42.87	70	178.77
80	48.25	80	185.43
90	53.91	90	191.93
500	59.86	700	198.26
10	66.06	10	204.41
20	72.50	20	210.36
30	79.13	30	216.12
40	85.95	40	221.66
550	92.91	750	227.00
60	100.00	60	232.11
70	107.18	70	237.01
80	114.44	80	241.67
90	121.73		

rapidly, and the assumed spectral energy distribution may become inaccurate.

Incandescent-filament lamp standards of color temperature are issued by the National Bureau of Standards at 2848°K. and at other color temperatures.

5·23 Spectral transmission standards. Reference has already been made (Section 5·16) to the glass standards of spectral transmittance issued by the National Bureau of Standards for checking the photometric scale of spectrophotometers. These standards have a high degree of permanence if the surfaces are kept undamaged and if they are not exposed to sunlight or other excessive radiant flux. However, they are not precisely reproducible from specification, and each glass must be independently calibrated before issuance. Accordingly no transmittance values for these glasses are tabulated.

However, the spectral transmittancies of certain solutions have been measured with sufficient care, and the solutions themselves are sufficiently stable, that they may be accepted as suitable standards for checking the photometric scale of spectrophotometers. These are:

1. The aqueous solutions of copper sulfate and cobalt ammonium sulfate used in the series of filters developed at the National Bureau of Standards for reproducing the colors of sunlight and daylight and for the determination of color temperatures (*20*).

The published values for absorbancy and transmittancy are given in Tables 5·4 and 5·5, together with the composition and certain other pertinent information. Many additional details are given in M114 (*20*), including the changes in absorbancy with temperature. Both solutions obey Beer's law over a considerable range. The values given for the eight mercury and helium wavelengths are considered the most reliable, with an uncertainty in A_s not exceeding 0.001 for the particular chemicals used. Spectrophotometric reproducibility of the chemicals is also considered in the paper. By increasing the thickness or concentration a wide range of the photometric scale can be covered, except at the shorter wavelengths.

2. An aqueous solution of potassium chromate, K_2CrO_4 (0.04 g./l.), in 0.05 N KOH. This solution has been studied by Hogness, Zscheile, and Sidwell (*59*) and others and is considered one of the most suitable as a standard of spectral transmittancy and absorbancy in the ultraviolet. Its absorption in the violet is also useful because the copper and cobalt solutions have too little absorption in this region to be of much value. Accordingly, an extensive series of measurements was initiated in 1940 by Mrs. Geraldine Walker Haupt of the Photometry and Colorimetry Section of the National Bureau of Standards to obtain

Table 5·4 Values of Spectral Absorbancy, A_s, and Transmittancy, T_s, for Standard Copper Sulfate Solution as Specified, Unfiltered, Thickness 10.00 mm., Temperature 25°C., Having the Following Composition:

Copper sulfate ($CuSO_4 \cdot 5H_2O$) *	20.000	g.
Sulfuric acid (specific gravity 1.835)	10.0	ml.
Water (distilled) to make	1000	ml.

Wavelength, mμ	A_s	T_s †	*Wavelength,* mμ	A_s	T_s †
350	0.0090	0.979	600	0.0680	0.855
60	.0063	.986	10	.0885	.816
70	.0046	.989	20	.1125	.772
80	.0035	.992	30	.143	.719
90	.0028	.994	40	.180	.661
400	.0023	.995	650	.224	.597
10	.0019	.996	60	.274	.532
20	.0016	.996	70	.332	.466
30	.0014	.997	80	.392	.406
40	.0012	.997	90	.459	.348
450	.0011	.997	700	.527	.297
60	.0011	.997	10	.592	.256
70	.0012	.997	20	.656	.221
80	.0014	.997	30	.715	.193
90	.0018	.996	40	.768	.171
500	.0026	.994	750	.817	.152
10	.0038	.991			
20	.0055	.987	Hg 404.7	.0021	.995
30	.0079	.982	Hg 435.8	.0013	.997
40	.0111	.975	Hg 491.6	.0019	.996
			He 501.6	.0028	.994
550	.0155	.965			
60	.0216	.951	Hg 546.1	.0135	.969
70	.0292	.935	Hg 578.0	.0368	.919
80	.0390	.914	He 587.6	.0487	.894
90	.0518	.888	He 667.8	.319	.480

* Analysis showed the copper sulfate to have 99.7 per cent of the theoretical copper content.

† Values of T_s are derived from values of A_s.

$$A_s = -\log_{10} T_s.$$

standard spectral transmittancy and absorbancy data for a specified potassium chromate solution of known stability. This work was interrupted before completion but sufficient data had been obtained that a tentative set of spectral transmittancy values could be adopted, and Mrs. Haupt has made these tentative values available for use in the

TABLE 5·5 VALUES OF SPECTRAL ABSORBANCY, A_s, AND TRANSMITTANCY, T_s, FOR STANDARD COBALT AMMONIUM SULFATE SOLUTION, UNFILTERED, THICKNESS 10.00 MM., TEMPERATURE 25°C., HAVING THE FOLLOWING COMPOSITION: *

Cobalt ammonium sulfate [$CoSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$] †	14.481	g.
Sulfuric acid (specific gravity 1.835)	10.0	ml.
Water (distilled) to make	1000	ml.

Wavelength, mμ	A_s	T_s	*Wavelength,* mμ	A_s	T_s
350	0.0038	0.991	600	0.0137	0.969
60	.0040	.991	10	.0124	.972
70	.0050	.989	20	.0115	.974
80	.0065	.985	30	.0112	.975
90	.0088	.980	40	.0110	.975
400	.0125	.972	650	.0105	.976
10	.0168	.962	60	.0097	.978
20	.0224	.950	70	.0087	.980
30	.0340	.925	80	.0076	.983
40	.0522	.887	90	.0066	.985
450	.0773	.837	700	.0054	.988
60	.1031	.789	10	.0046	.989
70	.1213	.756	20	.0038	.991
80	.1349	.733	30	.0032	.993
90	.1472	.713	40	.0030	.993
500	.1635	.686	750	.0028	.994
10	.1742	.670			
20	.1689	.678	Hg 404.7	.0144	.967
30	.1452	.716	Hg 435.8	.0437	.904
40	.1113	.774	Hg 491.6	.1497	.708
			He 501.6	.1661	.682
550	.0775	.837			
60	.0496	.892	Hg 546.1	.0901	.813
70	.0308	.932	Hg 578.0	.0219	.951
80	.0207	.953	He 587.6	.0167	.962
90	.0158	.964	He 667.8	.0089	.980

* These data apply accurately also from 400 to 750 mμ to a similar solution made up with 10.3 g. of cobalt sulfate ($CoSO_4 \cdot 7H_2O$).

† Chemical analysis showed a cobalt (plus nickel) content of 100.0 per cent of the theoretical, the ratio of nickel to cobalt (metals) being 1 to 200.

present chapter. They are given in Table 5·6. It is hoped that this work may be completed and final values published. Meanwhile, the following details are of interest and importance in connection with the use of Table 5·6.

TABLE 5·6 VALUES OF SPECTRAL TRANSMITTANCY, T_s, AND ABSORBANCY, A_s, FOR STANDARD POTASSIUM CHROMATE SOLUTION, UNFILTERED, THICKNESS 10.00 MM., ROOM TEMPERATURES (USUALLY CLOSE TO 25°C.), HAVING THE FOLLOWING COMPOSITION:

0.0400 g./l. of potassium chromate (K_2CrO_4) in 0.05 *N* potassium hydroxide solution *

Wavelength, mμ	T_s †	A_s ‡	*Wavelength,* mμ	T_s †	A_s ‡
210	0.000	......	350	0.28	0.5528
15	.037	1.4318	55	.202	.6946
20	.35	.4559	60	.148	.8297
			65	.115	.9393
225	.60	.2218	70	.102	.9914
30	.68	.1675			
35	.62	.2076	375	.103	.9872
40	.509	.2933	80	.118	.9281
45	.408	.3893	85	.152	.8182
			90	.207	.6840
250	.319	.4962	95	.30	.5229
55	.268	.5719			
60	.232	.6345	400	.41	.3872
65	.201	.6968	404.7	.52	.2840
70	.180	.7447	410	.635	.1972
			20	.748	.1261
275	.173	.7620	30	.824	.0841
80	.189	.7235	435.8	.861	.0650
85	.254	.5952	40	.884	.0535
90	.372	.4295			
95	.527	.2782	450	.928	.0325
			60	.961	.0173
300	.70$_5$	.1518	70	.981	.0083
05	.83	.0809	80	.992	.0035
10	.90	.0458	90	.998	.0009
15	.90$_5$	.0434	500	1.000	.0000
20	.867	.0620			
325	.810	.0915			
30	.71$_5$	.1457			
35	.60$_5$	.2182			
40	.48$_5$	.3143			
45	.38	.4202			

* This solution of potassium hydroxide can be prepared with sufficient accuracy by dissolving 3.3 g. of potassium hydroxide sticks (85 per cent KOH) of reagent quality in sufficient distilled water to make 1 liter.

† Tentative values. See text.

‡ These values of A_s derived from the values of T_s.

All the potassium chromate solutions were prepared by Mr. S. Clabaugh of the National Bureau of Standards Chemistry Division under the direction of Dr. E. Wichers. The data of Table 5·6 were obtained from solutions prepared in the following ways:

1. A solution of K_2CrO_4, stock material, reagent grade, 0.0400 gram per liter, in 0.05 N KOH.
2. A solution of K_2CrO_4 of the same concentration and alkalinity as (1) but prepared from 0.0303 gram of reagent grade * $K_2Cr_2O_7$, which when neutralized gave 0.0400 gram of K_2CrO_4 per liter.

Both solutions were found to remain stable (in transmittancy) for the 2 years during which the measurements were being made, if stored in ordinary storeroom glass bottles. "Flaking" may occur, and any sediment should be allowed to settle to the bottom of the bottle. It is recommended that alkali-resistant ware, now available, be used for storing the solutions.

The spectral transmittancy measurements were made by means of the following instruments and methods:

1. By photographic spectrophotometry with the Hilger sector photometer, on the equipment illustrated in Fig. 5·15 and described in reference (*47*).
2. By photoelectric spectrophotometry, using a double quartz monochromator designed by Brackett, Wensel, and Kuper (*7*) and at that time located in the Heat and Power Division of the National Bureau of Standards. Most of the work in the ultraviolet was done on this and the photographic spectrophotometers.
3. On a Beckman quartz spectrophotometer (*14*) in the *p*H Standards Section of the National Bureau of Standards. Only a few measurements were made on this instrument.
4. Above 400 mμ also on the General Electric recording spectrophotometer (*45*) and the König-Martens visual spectrophotometer (*81*). There is no measureable absorption above 500 mμ for this concentration.

All the measurements were made by Mrs. Haupt, assisted in some cases by Miss Margaret M. Balcom and Mr. Grant W. Holmes. Temperature control was not used, but room temperatures were usually close to 25°C. In the values of T_s given in Table 5·6 there is considerable uncertainty in the third decimal where three significant figures are used. Where but two significant figures are used, there is slight uncertainty in the last figure. These uncertainties are estimated from the degree of agreement of results by the different methods.

Further work contemplated includes measurements to be made on new solutions on the Beckman spectrophotometers in the Photometry and Colorimetry Section of the National Bureau of Standards with temperature control at 25°C. (*44*). These instruments had not been obtained at the time the above work was done.

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* Potassium dichromate of very high purity is available from the National Bureau of Standards, sample No. 136.

5·24 Spectral reflection standards. As a fundamental standard of spectral directional reflectance nothing has as yet been found more suitable than freshly prepared magnesium oxide. Its (total) luminous reflectance is high, 0.97 or 0.98, and nothing has been found of certainly higher reflectance. Its directional luminous reflectance, $R_{0,45}$, is 1.00, and its spectral selectivity throughout the visible spectrum appears to be less than 1 per cent. These data are based on work by Priest (*97*), McNicholas (*80*), and Preston (*94*) and are summarized in National Bureau of Standards *Letter Circular* LC-547 (*87*).

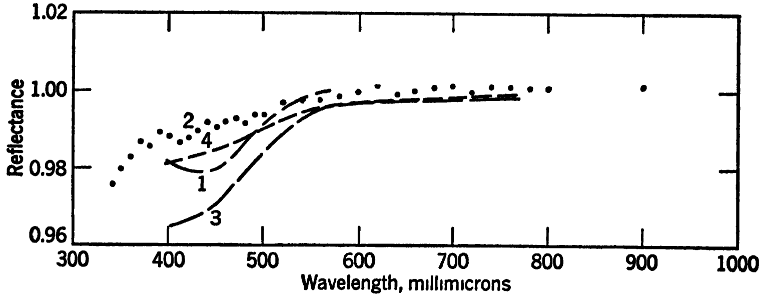

Fig. 5·33. Effect of age on the spectral reflectance of magnesium oxide. Values are plotted relative to the reflectance of freshly prepared MgO. Curves 1, 3, and 4 were obtained with the General Electric spectrophotometer, and curve 2 with the Beckman spectrophotometer. The ages of the samples were: curve 1, 2½ weeks; curve 2, 5 months; curve 3, 20 months; curve 4, 2 years. Obviously other factors than time also enter into the yellowing of MgO surfaces.

Although these characteristics make fresh MgO an excellent fundamental standard, it has other characteristics that are undesirable and that make the calibration and use of a secondary working standard a very advisable procedure. A MgO surface is extremely fragile and thus is not very suitable for continued handling. A more serious defect is that its spectral reflectance may change perceptibly within a short time (sometimes in a day) after preparation. The nature of the change is shown in Fig. 5·33; the reflectance decreases below 550 mμ as shown. Furthermore the nature and extent of the changes are variable. A third reason for use of a working standard is that slight variations in reflectance (0.1 or 0.2 per cent) have been noted for different preparations of freshly prepared MgO. By calibrating the working standard against several different MgO preparations a more representative standard is obtained than would be any single MgO surface by itself.

White structural glass by the name of Vitrolite, with polished surface, has proved the most suitable for working standards of spectral directional reflectance, although the material is not uniformly good for such purpose and must be selected with care. A considerable supply of suitable Vitrolite has been obtained by the National Bureau of Standards, and standards are now calibrated and issued for either the General Electric recording spectrophotometer or the Beckman quartz spectrophotometer.

H·ACKNOWLEDGMENTS

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6· PHOTOGRAPHIC METHODS*

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This subject has been ably reviewed, for instance by Brode (*4*) and Twyman and Allsopp (*37*), and the principles underlying photographic spectrophotometry have been discussed by Loofbourow (*23*). In this chapter the aim has been not so much to list the many technical devices which have been used for photographic measurement of absorption spectra, but rather to illustrate in fair detail the principles of one or two of the more generally used techniques which have proved satisfactory in the author's experience, to suggest possible lines of improvement, and to show the adaptability of photographic registration to different types of problems. Emphasis has been laid on sources of errors and on means for reducing them. Lastly a comparison is made of the relative merits of photoelectric and photographic methods. Descriptions of methods either unpublished or available only in less accessible texts are given in more detail than others.

6·1 The photographic process. The technique of photographic spectrophotometry, the design of photometers, and the accuracy of photometry are governed very largely by the properties of the photographic plate or film. The subject has been well reviewed on several occasions. For a more thorough study than is given here, the reader may consult the publications of Mees (*26*), Mott and Gurney (*27*), and Berg (*3*).

The steps by which the exposure of a photographic plate results in a stable black image may be summarized as follows: (1) formation of the latent image in the silver halide-gelatin emulsion; (2) development of the visible image as free silver; and (3) removal of excess silver halide. The optical density of the final developed and fixed image is the readily measured property which, in practice, is to be related to the energy of light to which the plate was exposed. This density is approximately proportional to the amount of silver in the emulsion. The size and shape of the silver grains are, however, also factors affecting the density.

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* Since this chapter represents English usage in terminology, no attempt has been made to have the symbols and terms consistent with those of *Letter Circular* LC-857, the standard adopted for the remainder of the book. The term *density* is widely used in this country, too, to refer to the degree of blackness shown in a developed photographic plate. The reader may keep in mind (see Table 2·1) that extinction coefficient, K, = absorbance index, a_i, and optical density, D or d, = absorbance, A_i. EDITOR.

6·2 The relation between density and exposure. The general character of the relation between the amount of the light falling on a photographic plate and the density of the resulting image is shown in Fig. 6·1.

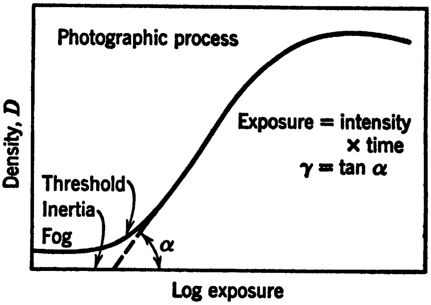

FIG. 6·1. The relation between exposure and resulting density of the photographic plate.

The form of the curve depends on the nature of the plate and developer, length of time of development, and wavelength of light.

The middle portion is often straight and can be fitted by an equation

$$D = \gamma \log It^p - i \qquad (6\cdot1)$$

where D is the density, I the intensity of the light, t the time of exposure, p the Schwarzschild (*35*) index, and i the inertia of the plate. The slope of the straight line portion, γ, is a measure of contrast.

Schwarzschild found p to be constant at a value of 0.8. His investigations, however, covered only the use of very low light intensities, such as occur in stellar photography. Later work has shown that, if the relation between D and log $(I \times t)$ is expressed in the form of equation (6·1), p is not constant, but varies according to the intensities of light used and with different types of plates from nearly 2.0 to as low as 0.40.

That p takes varying values, greater or less than one, expresses the fact that the photographic plate does not follow the photochemical reciprocity law of Bunsen and Roscoe (*6*), which states that the product $I \times t$, of intensity of light I and time of exposure t, will produce the same effect regardless of the values of I and t. A brief explanation, based on recent theory, of the reasons for the deviation from the law by the photographic plate is given below. The way in which the deviations from the law affect photographic measurements of light intensities in absorption spectrophotometry follows.

6·3 Reciprocity failure. If portions of a photographic plate be exposed to varying intensities of light and the duration of exposure be limited so that the product $I \times t$ be constant, the densities of the portions of the plate when developed will, in general, not be equal. It is found that there is a value of I for which the density is a maximum. In other words, there is an intensity of light for which the plate is maximally sensitive, the sensitivity falling off for higher and lower light intensities. It follows that we cannot deduce that two portions of equal density on a developed plate have been exposed to the same total light energy unless we have further information. Kron (*22*) was the first to give the form of the relation between light intensity and effectiveness of exposure. In Fig. 6·2 is shown a curve of this type.

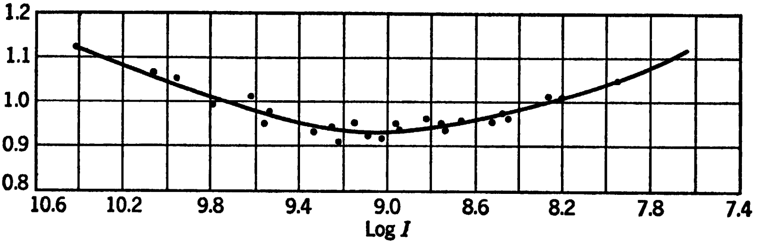

FIG. 6·2. Curve showing type of reciprocity failure for constant density.

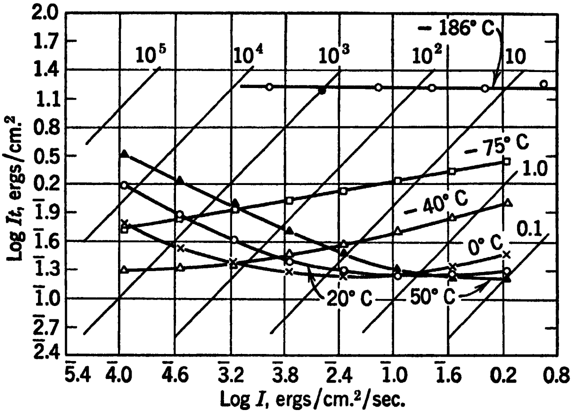

FIG. 6·3. Curves showing variation of reciprocity law failure with temperature. (Reproduced from *The Theory of the Photographic Process*, by C. E. K. Mees, The Macmillan Company, 1942, by permission.)

This form of expressing reciprocity failure is known as a constant-density curve. It demonstrates clearly the fact that, as the intensity of the illumination to which the plate is exposed varies, the total illumination ($I \times t$) required to produce a given plate density is not constant, which it would be if the reciprocity law held. Mees (*26*) states that the exposure intensity at which maximum efficiency occurs is about that value for which a medium density is produced in from 0.1 to 10 seconds.

Reciprocity failure shows marked variation with temperature. Figure 6·3, taken from Mees's book (*26*), illustrates this fact. It is to be noted that at −186°C. the reciprocity law is fulfilled. This is generally so for all emulsions. Further, at 20°C. there is, for the particular emulsion investigated, a hundred-fold range of intensity over which the law holds within narrow limits. Most emulsions show a flat minimum at room temperature, and, from a practical point of view, this may be important as a region where deviation from the reciprocity law is minimal.

6·4 Intermittency effect. The density of a developed photographic plate resulting from an exposure given in a number of separate installments (say by means of a rotating sector) may be greater or less than that resulting from a continuous exposure of the same total radiant energy. It is found that, if the speed of the sector is increased, that is, the installments and the intervals between them are made shorter, so does the density approach that which results from a continuous exposure of intensity equal to the time-average intensity of the discontinuous exposure. Equality is reached at sector speeds of about 300 revolutions per minute, but the speed needed varies with different plates.

The intermittency effect is an aspect of reciprocity failure. The extremes of the effect can be considered as due to the different effect of two light intensities, namely, on the one hand the intensity of the uninterrupted light and on the other hand the intensity of the light averaged over the time during which exposure is made through the rotating sector. The location of these two intensities on the reciprocity failure curve determines whether the intermittent exposure will have greater or less effect than the continuous exposure.

6·5 Explanation of the reciprocity law failure of the photographic plate. According to the theory first put forward by Mott and Gurney (*27*), the formation of the latent image is a two-stage process, the first electronic, the second ionic. It is therefore to be expected that the reciprocity law will not be followed, since this law is theoretically valid only for one-stage reactions.

1. *First stage.* It is electronic, very rapid, and insensitive to temperature, and its quantum efficiency is 1. This stage consists in the liberation of electrons from their stable orbits in the bromide ion. This implies a raising of the mean potential energy of the electrons in the nonconducting bromine lattice. The electrons move freely in the silver

lattice under the influence of any electrically polarized field until they are trapped by so-called sensitivity specks. These specks probably consist of minute particles of silver or silver sulfide. The specks become negatively charged by the liberated electrons. The electrons seem to be trapped more readily by specks situated on the surface of the silver halide crystal.

2. *Second stage.* It is much slower than stage 1 and is temperature sensitive. In this stage there is migration of silver ions from positions in the lattice to the negatively charged speck with consequent discharge of the speck and deposition of silver, which acts as the center for the action of the developing agent.

The explanation of the fact that the amount of silver laid down on the sensitivity specks in stage 2 is not proportional to the total light energy initiating stage 1 can be summarized as follows.

The negatively charged sensitivity specks are instable. There is definite leakage of their negative charge so that, for very low light intensities, the leak rate may be high compared with the charge rate (low-intensity reciprocity failure). The low speed of transfer of the positively charged silver ions to the negatively charged sensitivity specks may result in the speck acquiring such a high negative charge that it repels further electrons. These electrons may then be trapped by specks lying inside the silver halide crystals. The resulting silver aggregate is inaccessible to the usual developing agents. At high light intensities the silver ions may therefore fail to discharge the negatively charged surface specks at a rate sufficient to keep their potential lower than that which will force electrons into the specks inaccessible to developer (high-intensity reciprocity failure).

On the electronic scale any radiation must be considered as discontinuous, the energy being quantized. It is suggested that, when the exposing light is chopped by means of a sector at a rate equal to or greater than the mean rate of quantum transfer at a single silver halide crystal, then the photographic effect of the chopped light is equivalent to that of a continuous exposure of equal mean intensity. A consideration of the absolute energy of the exposing light in quanta per second and the cross-sectional area of the silver halide crystals in an emulsion render this explanation of the critical sector speed a plausible one.

6·6 Light sources. General considerations make it desirable that the luminous source should have a continuous spectrum and that the luminiferous area should approximate to a point, that is, taking the source as center, the distribution of light should be uniform over an arc as large as the aperture of the photometer (about 11° of arc in the case of the Spekker photometer).

It is now possible to construct light sources possessing these features and covering the range of sensitivity of the photographic plate. Figure 6·4 gives the approximate wavelength ranges for three such sources.

For nearly all purposes the early types of light source, such as underwater spark, Pointolite lamp, and high-voltage low-frequency hydrogen discharge in a long capillary, should become obsolete. The condensed spark between metal electrodes will remain as a point source for ultraviolet radiant energy until the new hot-cathode low-voltage short capillary hydrogen discharge has been perfected and tested. For the long ultraviolet, visible, and near infrared regions the ribbon-filament tungsten lamp fulfils the requirements very satisfactorily.

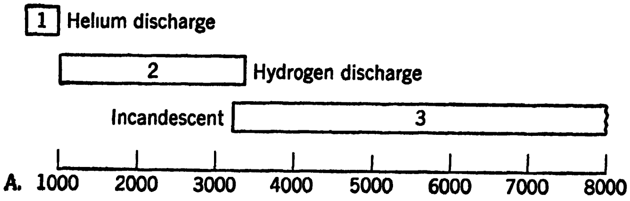

Fig. 6·4. The approximate spectral ranges covered by the continuous spectra of the electrical discharge in (1) helium, (2) hydrogen, and (3) incandescent tungsten.

6·7 The ribbon-filament lamp. This incandescent source has a filament 2 to 5 cm. long, 2 to 3 mm. wide, and 0.06 mm. thick. Operating at from 4 to 8 volts and 80 to 250 watts, it has a color temperature of 2400 to 2800°K. When viewed normal to the plane of the filament, it can be considered as a point source illuminant with a photometer of the type of the Hilger Spekker photometer, in which light for the two beams arises from the same point in the source. It may not be generally realized that the light emitted at an angle oblique to the face of the filament is partially plane polarized (*45*). When such a strip filament source is used with a photometer and spectrograph with quartz optical trains, trouble may be encountered from sets of interference bands which may appear in the photographed spectrum. These bands are due to interference between the ordinary and extraordinary rays which, after passing through the photometer, are polarized at right angles to each other and are out of phase owing to the different speed of light for the two rays in quartz. The phase difference will be dependent on wavelength. The oblique face of the spectrograph prism acts as *analyzer*, bringing the two rays to the same plane of polarization. Interference will then occur at those wavelengths at which the phase difference is an odd multiple of π. The effect can be demonstrated by setting up the strip filament lamp with its edge towards a spectrograph and taking an exposure with a quartz optical flat interposed between the lamp and the spectrograph. By adjustment of the exposure, the bands are easily recognized. The effect cannot be tolerated in absorption measurements using a split beam photometer, since the band systems may not be the same in the two beams, owing to the slightly different lengths of quartz traversed by the two beams. Also, when looking for fine structure in absorption spectra, the effect may cause considerable confusion. To avoid the effect the filament should be as wide as possible (>3 mm.) and should be mounted so that the optic axis of the photometer is normal to the plane of the filament. In this way maximal randomization of polarization of the radiant energy entering the photometer is obtained.

The strip-filament lamp can be used for spectrophotometry down to a wavelength of about 320 mμ. It is useful in the long ultraviolet range, for it gives a continuous spectrum in the region where the hydrogen discharge spectrum shows the Balmer series of lines.

6·8 The hot-cathode hydrogen discharge tube. In recent years the design of low-voltage hydrogen discharge tubes has been much studied, and Allen and Franklin (*1*) have constructed one which seems very well suited for use with ultraviolet photometers. It is fully described in their paper and is now commercially available. Suffice it to state here that the arc is concentrated into a space of maximum dimensions of 4 mm. Measurements of light distribution about the axis show that, with proper care, the tube can be constructed to give uniform distribution over an arc subtending 5° on each side of the axis. Although not originally designed as a source for a split beam photometer, it is well adapted for that purpose. It is important, however, to make quite certain that the aperture in the outer cylinder (anode) is symmetrically placed with respect to the aperture in the inner cylinder and large enough to allow unimpeded passage of light over a solid angle of 5° about the axis.

The arc runs at about 1.3 amperes at 80 volts. It operates on direct or alternating current and is very steady. The tube has a long life and is not expensive to purchase or run. It is very intense. When used with the Spekker photometer, exposures of only 3 times those required when using a 12,000-volt condensed spark across a 3-mm. gap are necessary. A modification of this tube giving still more intense radiant energy has been described in a further paper by these authors (*2*).

6·9 Condensed spark between metal electrodes. This has been and still is the most widely used source in photographic absorption spectrophotometry. The metal most preferred for the electrodes has been tungsten steel. With these electrodes the high intensity of the ultraviolet emission, the richness in lines and their fairly even distribution over the spectral range, the freedom from wandering, and the localization of the luminiferous area in a small space have made this source preferred for work in the ultraviolet. Its disadvantage is the discontinuous character of the spectrum. The intensity ratios of the strong and weak lines exceeds 10,000. These two properties make the spectral image unsuited to the detection of fine structure in absorption bands or to accurate determination of the wavelength position of match points with the microphotometer (see section on photometry). In spite of this disadvantage, the error of matching even a lined spectrum with a microphotometer is about one-third of that for visual matching.

6·10 The dispersing system. The choice of a suitable dispersing system for a photographic spectrophotometer is more restricted than in the case of its photoelectric counterpart. Three factors are involved.

1. A geometrical image of a slit has to be projected on a photographic plate. Only a portion of the resulting image will be used for densitometric comparison.

2. The slit must be uniformly illuminated. Some aspects of this requirement are discussed under photometers, sections 6·16 and 6·17. Ideally this condition requires that all that part of the slit which is to be used should be illuminated equally by all parts of the light source. This condition is most nearly attained when the slit is illuminated from a considerable distance without the intervention of a condenser, or when a condenser, placed immediately in front of the slit, focuses the enlarged image of the source on the collimator of the spectrograph. The illumination is much greater in the latter case.

3. A considerable range of the spectrum must be in focus on the photographic plate at the same time. Most spectrographs have a curved focal plane which entails the bending of the photographic plate. This procedure is never very satisfactory for accurate wavelength determinations. Apart from the nuisance of broken plates, the plate, even when gripped at both edges in templates of the correct curve, tends to be too flat in the middle, with resulting displacements of wavelength positions in different regions of the plate. Adam Hilger, Ltd., has marketed a prism spectrograph with a combination camera lens corrected to give an almost flat focal plane.

6·11 Stray light. Again, in absorption spectrophotometry, in contradistinction to emission spectrography, the dispersing system must be selected with a view to the minimization of stray light. This applies to both photoelectric and photographic methods. In absorption work measurements tend to be made in spectral regions where the light intensity is *reduced* as compared with that in other parts of the spectrum, whereas in emission work measurements are made in regions where the intensity is *higher* than in other parts. Consequently, absorption measurements are much more sensitive than emission measurements to interference by stray light from other spectral regions. With the increasing use of gratings for spectral dispersion, the fact is likely to be overlooked that, in spite of theoretical arguments to the contrary (*34*), such instruments may in practice give rise to considerable stray light especially in the short ultraviolet. Cary and Beckman (*7*) have shown that prism dispersion is much preferable to grating dispersion from this point of view.

In Table 6·1 are shown the relative intensities of stray light at various wavelengths of a typical grating and prism dispersing system, calculated from the results of Cary and Beckman. These authors state that, after testing eight different types of grating in three different types of optical mounting, they found no grating gave stray light less than 5 per cent of the monochromatic light.

TABLE 6·1 RELATIVE INTENSITIES OF STRAY LIGHT IN GRATING AND PRISM SPECTROGRAPHS

Calculated from Cary and Beckman (*7*)

Millimicrons	200	250	300	350	400	500	600	700	800
Grating	27	22	16	12.5	10	7	4.5	4.0	4.0
Prism	4.5	3.0	1.0	0.8	0.5	0.3	0.1	0	0
Ratio	6.0	7.3	16	15.6	20	23.3	45	∞	∞

Stray light interferes with photometric measurements, particularly in those regions of the spectrum where the response of the photographic plate or photoelectric cell is low compared with the response in other regions. For this reason, even using prism instruments, absorption measurements in the shorter ultraviolet (180 to 250 mμ) are liable to considerable error, especially if an attempt is made to measure high extinction coefficients.

It is of interest to calculate the effect on absorption measurements of the presence of a given amount of stray light. As an example, calculations have been made for the cases in which, at a given wavelength, 5, 3, and 1 per cent of the response of the plate or photocell to the direct light is contributed by stray light. These are graphically represented by the three curves in Fig. 6·5, in which, for each apparent optical density, the true optical density is plotted.

It is evident from the figure that the higher the optical density at which measurements are made the greater will be the error. The error

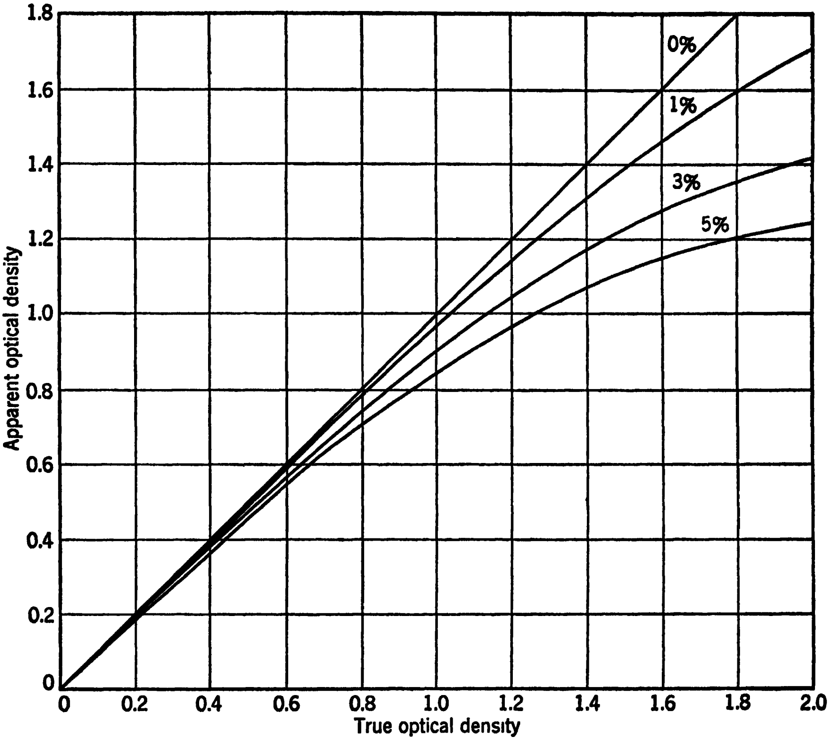

FIG. 6·5. The relation between apparent and true optical density calculated for different proportions of unabsorbed stray light in a monochromator source.

will always be negative, that is, the apparent optical density will be too low. In Table 6·2 the errors of measurement of some values of true optical density are tabulated.

TABLE 6·2 ERRORS OF MEASUREMENT OF TRUE OPTICAL DENSITY, CALCULATED FOR DIFFERENT PROPORTIONS OF STRAY LIGHT IN THE MONOCHROMATIC BEAM

(All errors are negative and are expressed in percentage.)

Proportion of Stray Light, per cent	True Optical Density											
	0.0	0.1	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	1.0	1.4	1.5	2.0	2.6	3.7	5.0	6.6	8.8	11.5	14.9
3	0	1.5	3.5	4.8	6.0	7.6	10.1	13.0	16.5	20.6	24.9	29.5
5	0	5.0	6.0	7.5	9.5	12.2	15.5	19.3	23.7	28.3	33.2	37.8

6·12 Resolution. No general study of the resolving power required in spectrographs to be used for spectrophotometry has been recorded. It is generally accepted that these requirements are far less stringent than for emission work. Most commercial spectrographs have a resolving power in excess of that required for absorption work.

Linear dispersion is a more important consideration. If $d\theta/d\lambda$ be the angular dispersion of a 60° prism, the linear dispersion ($dx/d\lambda$) is $f\, d\theta/d\lambda$, where f is the focal length of the camera lens. On the other hand, the resolving power $\lambda/\Delta\lambda$ can be shown to be the product of the length of the prism base, B, by $d\theta/d\lambda$, that is, $B \times d\theta/d\lambda$, where B and λ are expressed in angstrom units. $\Delta\lambda$ is the length, in angstroms, to be used as a criterion of the resolution of two lines of slightly different wavelength and is the width of the central diffraction maximum of the slit of the spectrograph (Rayleigh's criterion). It can be shown that the width of the diffraction maximum is proportional to the focal length of the camera lens. Hence, by increasing the focal length of the camera lens, no increase of optical resolving power will result. On the other hand, the linear dispersion will be increased in proportion. Now, if we consider the operations entailed in spectrophotometry, it will be evident that the error of "placing" a match point will be inversely proportional to the linear dispersion of the spectrograph. The linear resolution of a microphotometer, that is, the error with which a match point can be "placed," is about 0.1 mm. under the very best conditions, using a microphotometer and a continuous spectrum. In the case of the steepest absorption bands, for example of polycyclic hydrocarbons, the optical density (D)/wavelength slope may reach 0.1 D per angstrom. Since, for most spectrograms, intervals of 0.1 D are sufficiently small, we obtain the criterion of dispersion that 1 A. should be equivalent to 0.1 mm. on this plate, that is, 1 mm. per A. dispersion. For a medium-sized quartz spectrograph with a dispersion of about 23 cm. for the range 2000 to 10,000 A., this dispersion is reached only below 2700 A. At about 3500 A., where these extremely sharp bands occur, such a spectrograph has only about one-third the dispersion required to satisfy the criterion.

Although for most purposes such a spectrograph has ample dispersion, this example has been given to illustrate the necessity for perhaps revising the prevalent view that dispersion is a secondary condition for absorption spectrophotometry.

6·13 Combination of photometer and spectrograph. In choosing a combination of photometer and spectrograph it is essential to see that the collimator lens of the spectrograph accepts the whole beam from the photometer apertures. This will have been provided for by manufacturers of the combination but should be remembered when combining a photometer and spectrograph not designed to work together. If this condition is not fulfilled, the collimator will act as a partial stop to one or both of the photometer beams and cause an apparent inequality in the two beams. It can be tested by placing a photographic plate just in front of the collimator and exposing it to the full light of the two beams of the photometer. The resulting blackened area should fall well within the circle representing the projection of the collimator on the photographic plate, placed in the position described.

6·14 Spectrophotometers.* From a consideration of the relation between exposure and blackening of the photographic plate previously discussed, it is apparent that, for two small areas of such a plate (A and B) of density D_a and D_b produced by light energies ($I_a \times t_a$) and ($I_b \times t_b$), the most accurate estimate of the relative light intensities I_a and I_b will be obtained when $D_a = D_b$, and $t_a = t_b$. Furthermore, the areas A and B should be close together on the plate to avoid effects of unequal development (see Section 6·17 on development). Under these conditions in the equation

$$\frac{D}{\gamma} = f(I \times t) \tag{6·2}$$

the estimate of $I_a/I_b = 1$ will be independent of the form of the function relating exposure to blackening of the plate. In other words, comparison of two light intensities is made most accurately photographically when the intensities are equal. Modern photometers are designed to fulfill these conditions. The principle of measurement consists, first, in varying continuously or discontinuously the ratio of light falling on two neighboring portions of a photographic plate and, second, by comparing the resulting pairs of plate densities, in determining that pair which is matched. (See sections on matching.) Under favorable circumstances the *light intensities* may be compared with an error of 0.01 per cent. However, in spectrophotometry, matching of radiant energies of homogeneous wavelength composition, that is, monochromatic radiant energy, has to be made. The complication involved in the optical train required to bring this about introduces systematic errors into the measurement which prevent the attainment of this accuracy.

6·15 Design of photographic spectrophotometers. The common feature of all such instruments is that two spectra originating from the same source are photographed on the same photographic plate.

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* See Chapter 5, Section 5·12, for a brief statement on the photographic equipment at the National Bureau of Standards.

One spectrum originates from the light beam which has passed through a cell containing solvent and the other from the light beam which has passed through a cell containing the solution of the substance under examination. The former or "control" beam can be varied in intensity. The amount by which the intensity of the control beam must be reduced to give the same blackening of the plate as that given by the "solution" beam is a measure of the amount by which the solute has reduced the intensity of the solution beam.

Because of the failure of the plate to obey the reciprocity law of Bunsen and Roscoe, older methods, such as those of Henri (*13*) in which reduction in intensity of the control beam was virtually obtained by a reduction in exposure time, have given place to methods in which time of exposure is the same for each beam (see below). Because spectrographs had been brought to a high state of perfection before the introduction of quantitative methods of spectrophotometry, spectrophotometers have usually been built up by the addition of a photometer to a spectrograph. This is the standard form of spectrophotometer. A notable exception is a spectrophotometer designed and built by Philpot and Schuster (*32*), in which the light-measuring and dispersing devices are, in both principle and construction, one unit. The instrument has not been produced commercially, but one is in operation at the National Institute for Medical Research in London. A very complete description has been given (*32*).

The basic design of most modern photometers used in absorption spectrophotometry is due to Twyman (*20*, *38*) and was embodied in the Hilger sector photometer and in that of Keuffel and Esser.

6·16 Sector photometer. Immediately in front of the slit of a spectrograph is a biprism *B* (Fig. 6·6) which receives light from the source *L* by two alternative paths. An upper beam passes through a cell *A* containing solvent and the wedge prism *P*. The beam then passes through a rotating sector *S*, the aperture of which can be varied, and, falling on the biprism *B*, is deviated by the lower half of that prism to pass axially along the collimator of the spectrograph. The second beam traverses first the absorbing liquid under examination, contained in a cell *A′*, then through the wedge prism *P′*, similar to *P* but so arranged as to divert the light upwards instead of downwards, and a rotating sector *S′* of fixed aperture. The second beam is then diverted by the upper part of the biprism *B* and passes axially along the collimator of the spectrograph like the first. The spectrograph is therefore illuminated by two beams, the one capable of being varied in intensity at will by varying the aperture of the rotating sector, the other subject to absorption of known thickness of the liquid under examination.

Two criticisms have been made of this instrument: first, the intermittency effect might be likely to cause error; and second, the prismatic lenses, P and P', converge light on the spectrograph slit in such a way that adjacent portions of the resulting spectrum pairs originate in more or less widely separate points in the source.

The first objection has been repeatedly rebutted (*20, 24, 44*). Later O'Brien and co-workers (*29, 30, 31*) and Webb (*43*) showed that, with high enough sector speeds, depending on the make of plate, the intermittency effect disappeared completely (see Section 6·1 on the photographic process).

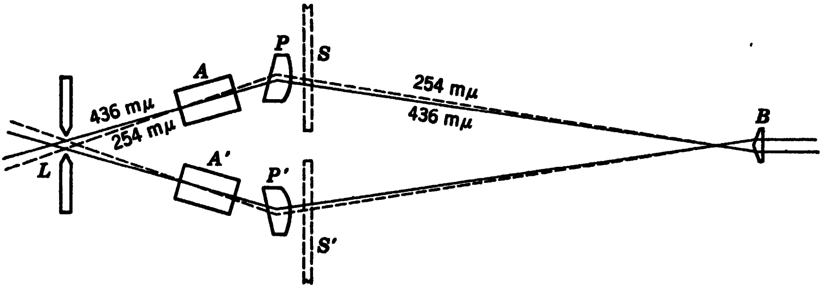


Fig. 6·6. Diagram of the optics of the Hilger sector photometer, illustrating the chromatic aberration due to the prism lenses.

The second objection is more serious and finally led Twyman to design a second photometer in which both these defects were eliminated. The Spekker photometer made by Adam Hilger, Ltd., embodies this second design (*39*).

6·17 Variable diaphragm photometer (Hilger Spekker photometer). The light from a suitable point source of ultraviolet rays A (Fig. 6·7) passes toward the inner edges of two quartz rhombs C from which the beams are diverted upwards and downwards, each to be then reflected forwards through an aperture, the upper one fixed, the lower one variable, by means of a micrometer screw D. The lenses L collimate the beams, which then pass through the absorption cells F (solvent only in the lower, solution in the upper) to be focused via the rhombs H by the lenses G just beyond the slit of the spectrograph in such a way that in the focal plane the image formed by the top, together with that formed by the bottom, lens forms a complete image of the light source.

Several advantages of this system over the former one may be noted.

1. The light for each beam is collected from a more nearly similar aspect of the light source.

2. The aperture of the instrument is larger.
3. Closely adjacent points on each side of the dividing line between the spectrograms receive light from closely adjacent points in the source.
4. Since the light is not interrupted, there is freedom from any possible intermittency effect. Also, exposures are shortened.
5. The density scale on the drum is more open and can be set more accurately. Higher density settings are possible.

The Spekker photometer is probably the most satisfactory photometer available for photographic absorption spectrophotometry. Its disadvantages are (1) the number of quartz-air faces liable to collect dirt, (2) the difficulty of cleaning, (3) the rather short focus, which makes the light-source position critical, and (4) the length of quartz in the optic train, which prevents its use below 220 mμ.

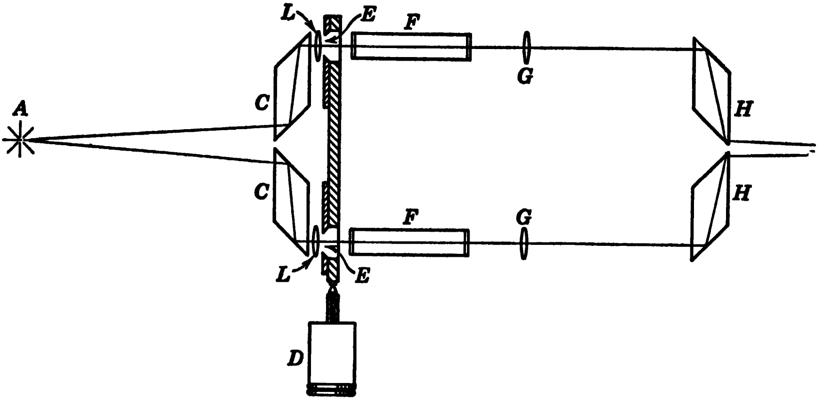


FIG. 6·7. Diagram of the optical system of the Hilger Spekker photometer.

6·18 The Philpot-Schuster spectrophotometer. Here the design is radically different, both the solution and the photometric aperture being placed between the defining slit of the spectrograph and the prism. Since the original account may be difficult to obtain, a rather full description is given here of the apparatus, the principles of which deserve careful consideration. A spectrogram obtained with this apparatus is illustrated in Fig. 6·8. Light reaching A has passed through the cell containing the absorbing liquid, whereas light reaching region B has not. All light has passed through the logarithmic stop $S3$ and cylindrical lens $L3$ (Fig. 6·9) arranged so that the aperture decreases exponentially from top to bottom of the spectrogram. Consider two points a and b in regions A and B on the same spectral line, and distance x apart. The ratio of the aperture at a to that at b is 10^{kx}, where k is independent of x. If there were no absorption, this would also be the ratio of the intensities of illumination (provided the illumination is uniform over the whole aperture); but since I_a has been reduced by absorption, the actual ratio of intensities is:

$$\frac{I_a}{I_b} = 10^{kx} \times 10^{-(d+d_0)} = 10^{kx-(d+d_0)} \ldots \tag{6·3}$$

where d is the optical density of the solute, and d_0 the optical density of the cell and solvent (measured separately).

If x is chosen so that $kx = d + d_0$, the intensities I_a and I_b will be equal, and points a and b will be points of equal photographic density. For this value of x, $d = kx - d_0$. Therefore, d can be found by measuring the distance apart of two points of equal density on the same spectral line, one in region A and the other in region B, and subtracting the result of a similar measurement made with the cell filled with solvent. This is the principle of the wedge spectrogram (*10*, *25*, *28*). The measurement of x is made by means of a specially designed densitometer which is fully described in the paper (*32*).

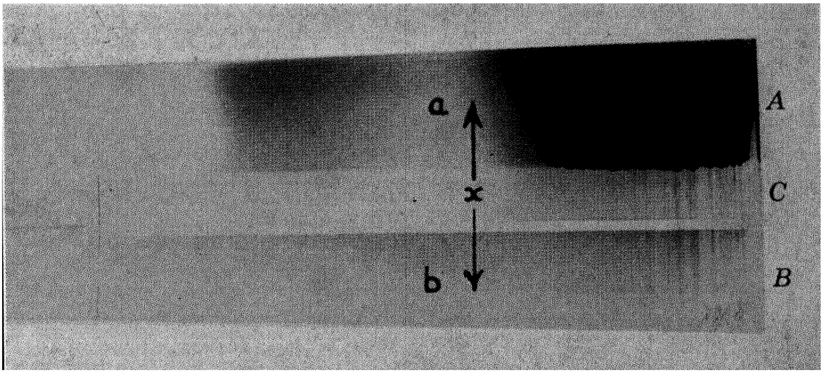


FIG. 6·8. Spectrogram obtained with Philpot-Schuster spectrophotometer. (Courtesy of the Controller, H.M. Stationery Office.)

Figure 6·9 shows the optical system of the spectrograph and its outlying parts. The horizontal scale of feet shows the distances between lenses, while the vertical scale of inches shows their aperture. The relative aperture is thus exaggerated ten times.

The light source, H, is an end-on hydrogen discharge tube with capillary approximately 10 cm. long and 2 mm. in diameter. The lens $L1$ focuses the beam at an intermediate wavelength on a short coarse slit, $S1$, 4 mm. high by 0.5 mm. wide, which acts as a secondary source. A roughly logarithmic stop in front of C (not shown) defines the aperture in a preliminary way. The absorption cell *C* consists of two quartz end plates clamped against a quartz spacer (see Fig. 6·10). The upper part of the light path goes through the liquid, and the lower through an equal thickness of quartz. The angular aperture in this region of the light path is so small that the light going through the cell is practically parallel. The position of the cell is such that it is focused on the plate in the vertical plane, as shown by the dotted line in the elevation.

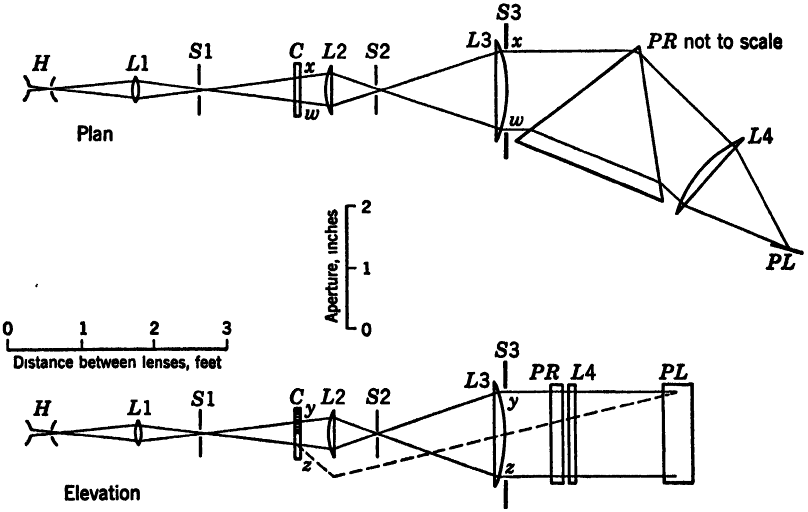
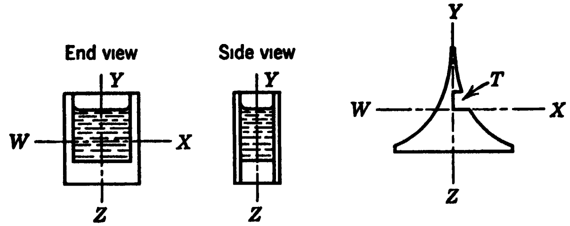


FIG. 6·9. Diagram of the optical system of the Philpot-Schuster spectrophotometer. (Courtesy of the Controller, H.M. Stationery Office.)



The cell

The logarithmic stop

FIG. 6·10. Diagram of the cell and logarithmic stop of the Philpot-Schuster spectrophotometer.

This gives a sharp division between the two portions of the spectrogram and also minimizes the effect of optical imperfections in the cell. The lens *L2* forms a reduced image of *S1* at the spectrograph slit *S2*. *S2* does not define the light path, but acts merely as a stop against stray light. The lens $L3$ is the collimator of the spectrograph. $S3$ is an accurate logarithmic stop which finally defines the aperture. The width y at a distance x from the top is given in millimeters by $y = 10^{0.04924x}$, where x is from 0 to 32.5 and y from 1 to 40. The shape is shown in Fig. 6·10, which also shows a tongue T, 6 mm. high, projecting inwards to the middle line a little more than half-way from the bottom of the stop. The position of the dividing line between quartz and liquid in the cell C is adjusted to correspond with the top of the tongue T. The effect of the tongue is to halve the aperture of a portion of the beam which has gone through the liquid. Therefore, in the absence of absorption, points of equal density on the spectrogram will occur at a distance apart corresponding to a fictitious absorption $I_0/I = 2$. This distance must be subtracted from the observed distance apart to obtain that due to genuine absorption. In this way the points of equal density are placed far enough apart to avoid the dividing line between the two beams even when the absorption is zero. For measuring higher absorptions the region of full aperture below the tongue is used.

The prism PR is of the usual Cornu type. The lens $L4$ is cylindrical with axis vertical. Light of a given wavelength is thereby focused to a vertical line, and the intensity at any point on the line is proportional to the width of the logarithmic stop at the corresponding height, provided the light is uniformly distributed over the aperture of the stop. The logarithmic relation described in the introduction is thus obtained.

The unusual positions of the logarithmic stop, cylindrical lens, and defining slit $S1$ give the following advantages:

1. Curvature of spectral lines and effects of nonuniform slit width are entirely avoided, since every part of the slit contributes to every part of a spectral line.
2. The logarithmic stop is at the widest part of the light path, so that the mechanical accuracy demanded of it is not excessive.
3. At the same time, the relative aperture of the lens $L1$ is very small. This allows the secondary source to be defined at $S1$ without loss of uniform distribution of intensity over the whole aperture for a wide range of wavelengths, even though $L1$ is not achromatic.
4. The angular aperture of the light path as it emerges from the hydrogen tube is also small. This is important, owing to the fact that the light comes from the whole length of a narrow capillary.
5. However small the aperture or the source, the whole of the diffraction pattern reaches the plate. With very small apertures some light is diffracted to points corresponding to adjacent wavelengths; but the amount so lost is compensated by the gain from other wavelengths if the second differential of intensity by wavelength is small, as in the

continuous hydrogen spectrum. The overlapping of adjacent wavelengths for small apertures does not affect the resolving power for absorption bands, since light passing through the region of small aperture has not passed through the absorbing liquid.

6. The quantity k in equation (6·3) varies much less with wavelength than is the case in most wedge spectrograms.

PHOTOMETRY

6·19 Density of a plate. The optical density, D, of an absorbing material is defined as being equal to the logarithm of the ratio of the intensity of the incident light, I_0, to that of the transmitted light, I:

$$D = \log_{10} \frac{I_0}{I}$$

In practice, as applied to a photographic plate, the conditions under which this is measured must be defined according to whether the scattered transmitted light is to be included in the value of I. In the applications described in this chapter it is usual to define I as excluding all but very small angle scattering. This satisfies conditions in which a plate is visually inspected or measured with a photoelectric microphotometer.

Matching is made visually or by means of a photoelectric microphotometer.

6·20 Visual matching. *a. By direct inspection.* The best viewing conditions vary somewhat according to the size of the image and the nature of the spectra. It is best to place the plate over an aperture in a light-tight box in which a light source is placed so that the plate is illuminated indirectly by reflection from a white mat surface. It is essential that the illumination be quite uniform to the eye. Any patterning of light and shade on the reflecting surface adds to the strain of viewing, even if it does not contribute appreciably to errors of matching. It will be found that there is an optimal illumination intensity for ease of matching. A rheostat in series with the lamp enables adjustment of the light intensity while viewing. Some portions of lined spectra, where the lines are very dense, are particularly difficult to match. A bright light and a magnifying glass giving magnification ×3 make this matching easier. According to the nature of the spark source, the make of plate, and the kind of developer, the lined spectra will show more or less background image. If the spectra are matched by naked eye, there is a tendency to match on this background by a process of lateral discrimination (see Fig. 6·11). If, on the other hand, the spectra are viewed through a magnifier, matching is more likely to be made on the spectral lines by vertical discrimination (see Fig. 6·12). In principle it is more reliable to match on the lines than on the background. A proportion of the background density is contributed by stray light of heterogeneous wavelength. This proportion is likely to be higher in the background than in the line blackening; there is, therefore, greater risk of error by matching on the background. Statistical tests have shown that the eye tends to give preference of blackening to the lower of a pair of adjacent spectra (*15*). This fact is revealed quite clearly by matching a plate one way up and then inverting it and matching again.

FIG. 6·11. Spectrogram obtained with Hilger Spekker photometer with hydrogen tube as a light source.

Match points are usually marked by placing an ink dot just below the lower of the two spectra at the wavelength at which the spectra appear to match. If this dot is placed on the gelatin face, it is difficult to remove and biases any subsequent matching. If the match point is marked on the glass side of the plate, it can easily be wiped off and the biasing of subsequent matchings is avoided. This method is recommended. Care should be taken, however, to avoid parallax.

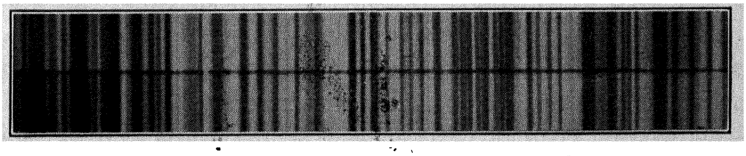


FIG. 6·12. Same as Fig. 6·11, but using a spark source.

The wavelength of the dots is best read by means of a fine cursor line engraved on glass or transparent plastic supported so that it lies parallel to the spectral lines and wavelength scale divisions. Arrangements should be made to enable the line to be moved along the spectrum and rotated relative to the plate. By this rotation the cursor is set to cut the wavelength scales printed at the top and bottom of the plate at the same wavelength reading. The cursor is then moved to coincide with each match point in turn, and the wavelength is read off from the scales.

Where the spectra originate from a continuous source such as a hydrogen tube or underwater spark, the ambiguity of line or background matching is absent. The density differences in different parts of the spectrum are not extreme as in spark spectra, and the need for varying intensity of illumination is scarcely necessary. The discrimination is made in the lateral direction along the pairs of spectra. It is necessary to be able to view the spectra at rather wide ranges of magnification so that the rate of change of density in the lateral direction will be fairly steep. A compromise has sometimes to be made between the high density gradient obtainable and the accuracy with which a match point can be placed and read off on the wavelength scale. The precise conditions seem to be a matter for choice by the particular observer. Continuous spectra should always be spotted on the back, for they are well suited to repeat matching. Such repeat matchings are well randomized, as the spectra are free from any localizing features that the eye may recognize.

b. By enlargement and projection. The plate is placed in an enlarger and the image projected at a magnification of 4× to 10×. The enlarger must be able to cover the vertical dimension of the plate to include both wavelength scales and spectra. By traversing the plate in the holder the whole length of the spectrum can be covered. Spotting and reading off are made on the screen with pen and ruler, care being taken that the ruler cuts the wavelength scales at identical readings. The advantage of this method is that repeat matchings may be made without bias. It is more cumbersome and expensive than the method of direct inspection.

6·21 Matching with a microphotometer. This method has not found such extensive use in absorption as in emission spectrography. There are several reasons for this, chief among which is the opinion that the over-all error of measurement of absorption is little reduced by matching with a microphotometer rather than visually, since the matching error contributes but a small fraction of the over-all error. Furthermore, it is argued, where matching is to be made on lined spectra the use of the microphotometer is laborious. In the discussion on errors the truth of the former statement is questioned. The method has been used with considerable success for some years in the author's laboratory. It is true that the procedure is laborious when measuring on discontinuous spectra; but with the introduction of the point-source hydrogen discharge tube and the consequent availability of continuous spectra, this disadvantage almost entirely disappears. In fact, finding the match point of a pair of continuous spectra by microphotometer is much easier than by eye, especially where the "cross over" is not sharp.

The microphotometer that should be used is of the concentrated beam type. In such an instrument the reduced image of the filament of a straight filament light source is focused as a line on the plane of the plate emulsion by means of a cylindrical lens and a microscope objective.

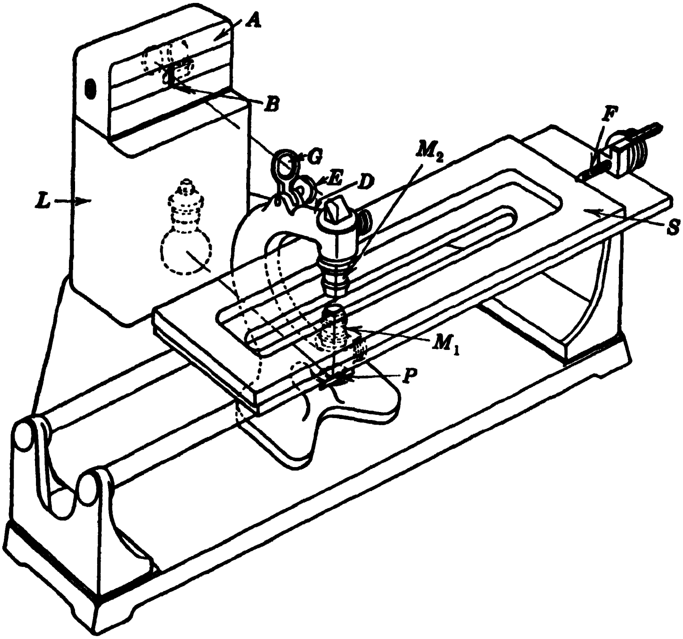


FIG. 6·13. Diagram of the Hilger microphotometer for matching plates.

By means of another microscope objective the magnified image of the pair of spectra is focused on a slit in the housing of two photocells. One of these receives the light from one spectrum; the other, by way of a deflecting rhomb, receives the light from the other spectrum.

Such an instrument is made by Adam Hilger, Ltd. Its general construction is shown diagrammatically in Fig. 6·13.

A prism at P deflects the beam from a light source at right angles on a 25-mm. microscope objective below the stage; this, together with a cylindrical lens (not shown), placed between L and P, forms an image of the filament of the source on the plate. The linear dimensions of the image are one-tenth of those of the filament. A second objective M_2 forms an image of the plate on the slit B, which is in the center of screen A. The magnification of this system is 10×. The light passing through the upper half of the slit *B* is separated from that passing through the lower by means of a rhomb placed behind the lower half of the slit. By this means the light from each of a pair of adjacent spectra is made to illuminate one of a pair of barrier-layer cells connected in opposition to each other and in series with a low-resistance short-period galvanometer.

By means of an equalizing glass *G* the illumination of the two cells can be balanced through a portion of the clear glass of the plate. At the point of balance there will be zero current through the galvanometer. Once the illumination to the cells is equalized in this way, they will remain so for a considerable time, depending on the differential variability of sensitivity of the cells due to fatigue or other reasons. Provided the spectral composition of the light arriving at the cells remains constant, this zero setting will hold for the widest range of light intensities likely to be used in matching plates. The finding of a match on a pair of adjacent spectra, after proper alignment of the plate, is a matter of finding that point at which a zero deflection of the galvanometer is obtained.

The chief sources of error in using the instrument can be ascribed to (*a*) variation of the spectral composition of the light falling on the cells, and (*b*) incorrect alignment of the plate and focusing.

There is in practice always a difference in spectral response between pairs of barrier-layer cells. Therefore, wide variations in the intensity of the light source with consequent variation in spectral composition should be avoided. It has been found that, with a randomly chosen pair of cells, limitation of intensity variations of light source up to 10 per cent insures freedom from this error. The developed photographic image is not a neutral gray so that a change of spectral composition of transmitted light accompanies change in density. This is not a serious source of error with a plate developed with hydroquinone and free from staining.

If such errors are suspected, a test can be made by examining in the microphotometer a graded optical wedge made on a photographic plate by the following method. A plate is exposed in the spectrograph, with a long slit illuminated by a distant source of continuous spectral characteristics, for example, a hydrogen discharge or incandescent filament. The resultant spectrographic image shows a smooth density gradient along the wavelength axis. The processed plate may now be scanned simultaneously by both photocells in the microphotometer from clear glass to the highest densities usable. Errors due to departure from neutrality of the developed image are detected by a disturbance of the balance with the variation of density. The only cure for any detectable error arising from this cause is to procure a more nearly matched pair of cells.

Correct alignment in the microphotometer is attained when there is parallelism of the line cutting the wavelength scale printed at each edge of the plate, the images of the spectrum lines, the longitudinal traverse of plate holder, and the slit of the cell housing. Furthermore, the lateral traverse of the plate holder should be parallel to the dividing line between the spectrum pairs. This obviously means that the images of the spectrum lines must be normal to the dividing line, a condition that is obtained by adjustment of the orientation of the slit of the spectrograph. A microphotometer should be fitted with adjustments by which these conditions may be satisfied. Alignment of the spectrum line images with the slit of the microphotometer is made by orientating the plate so that, with the two cells equalized on clear glass, there occurs minimum galvanometer deflection on traversing from one side to the other of a pair of lines judged to be of equal intensity at their centers. These conditions are fairly exacting when measuring on discontinuous spectra but may be relaxed as regards the last alignment when measuring on continuous spectra.

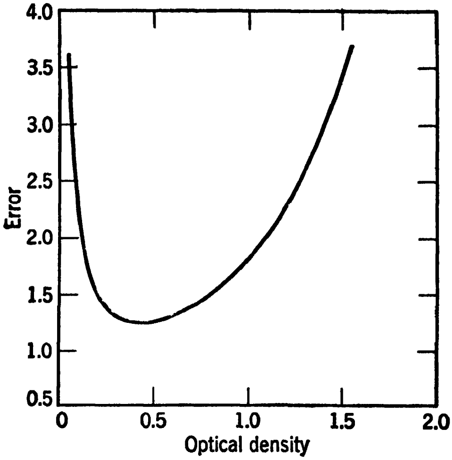


FIG. 6 · 14. The error in measurement of optical density, $\Delta D/D$, plotted as a function of a constant error, ΔI, in the measurement of light intensity, I (see refs. 23 and 37).

The sensitivity of matching with a microphotometer varies with the optical density of the matched areas. It can be shown that there is an optimal density at which, for a given deflection of the galvanometer or recorder from zero, the difference in density of the two spectra is minimal (*37*). This density has the value 0.4343. On each side of this value the minimum density difference which can be detected increases, as shown in Fig. 6·14. The unequal wavelength distribution of energy from light sources and the unequal wavelength response of the photographic plate make it impossible to measure at all wavelengths at this optimal density. Moreover, at this low density of $D = 0.43$, the graininess of the plate begins to cause erratic results. In a lined spectrum the density range of the line images is very great, and it is necessary to match on selected lines of a suitable density. In the continuous spectrum of the hydrogen discharge tube it should be possible to adjust exposure of the plate so that the whole ultraviolet spectrum lies within a range of density for which the microphotometer is working near its optimum sensitivity. For instance, using a Hilger medium spectrograph and a Bey and Steiner hydrogen discharge tube, an exposure could be found to give on a Kodak B10 plate the following densities:

Millimicrons	390	370	350	330	310	290	270	250	230
Densities	0.91	1.26	1.28	1.17	1.01	0.91	0.81	0.76	0.44

From Fig. 6·14 it can be seen that at the maximum density (1.28 at 350 mμ) the microphotometer will be two and a half times less sensitive than at the minimum density (0.44 at 230 mμ).

6·22 Processing. The processing of plates is well described in textbooks on the subject. Emphasis should be laid on two points.

1. With match-point methods, the aim should be maximum contrast, γ. Caustic hydroquinone developers are best for obtaining high γ. Eastman or Kodak D8 is a very useful one-solution developer for this purpose.

The sensitivity of measurement of the optical density, d, of a solution will be proportional to the γ of the plate used, for

$$d = \log \frac{I_0}{I}$$

and on the straight portion of the plate response curve

$$D = \gamma \log I$$

where D is the plate density, I is the intensity of the light falling on the plate, and d is the optical density of the solution.

Hence

$$\frac{\Delta d}{\Delta D} = \gamma$$

It is therefore desirable to choose a plate and method of development to give the highest γ. For use in the ultraviolet, the Ilford process plate and Kodak B10 photoscript plate developed in caustic hydroquinone show the highest γ (see Fig. 6·15).

2. With other methods, such as the Philpot-Schuster (*32*) or the Holiday cam method (*14*), even development is a prime requisite. Various procedures have been described (*26*). The continual agitation and replenishment of developer at the plate surface are sought in all these methods. There is no doubt that, for ordinary work, wiping the plate continually with a broad soft brush during development in a dish gives the most even development. This has the great advantage of avoiding the use of large amounts of developer. Several machines have been devised for agitating the developer during development (*8*, *12*, *36*). Below is described a hitherto unpublished method devised by R. Schuster of the National Institute for Medical Research, London.

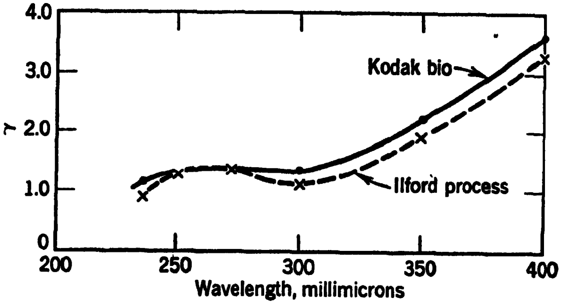


FIG. 6·15. Variation of γ (time scale) with wavelength (Kodak D8 developer; 1 minute at 20°C.).

In the tank (see Fig. 6·16) the plate or plates (1) are held face downward below the surface of the developer and a paddle (4) below them is driven backwards and forwards from end to end of the tank at a constant speed. The top edge of the paddle is brought as near as practicable to the face of the plates.

The plates are held in grooves cut in two vertical strips of Bakelite (2), the upper edges of which are attached to the inner cover (3) of the tank. This is provided with handles by which it can be lifted out of the tank after the outer cover (7) has been taken off, when it is required to insert or remove plates. The paddle (4) is mounted on an arm (5) bored at its upper end to take the push rod (6), to which it is rigidly attached. The push rod is long enough to extend beyond the tank at both ends, whatever the position of the paddle. It passes through packed glands in the end plates of the tank. The motion of the paddle is conveyed to it through the push rod, which in turn receives its own motion in the following manner.

A length of bicycle chain (8) is carried on two sprocket wheels (9, 10) suitably pivoted. The axle of wheel (9) also carries another wheel through which, and other gearing, it is driven by an electric motor at such a speed that the chain makes 12 complete circuits in a minute. The coupling pin of the chain projects outwards beyond the links to form a stud (11), which enters the slot (12) in the transverse member (13) fixed to one end of the push rod (6). When the chain is traveling as shown by the arrows and the stud is on its upper horizontal stretch, the push rod, and with it the paddle, will be forced towards the right at a constant speed. When the pin passes over the center of sprocket (10), it will begin to pass downwards in the slot and the speed of the push rod will decrease harmonically to zero, then reverse and increase again until the point below the center of the sprocket is reached. The motion will then become constant in a left-hand direction. It will be reversed again in a corresponding manner when the sprocket (9) is reached.

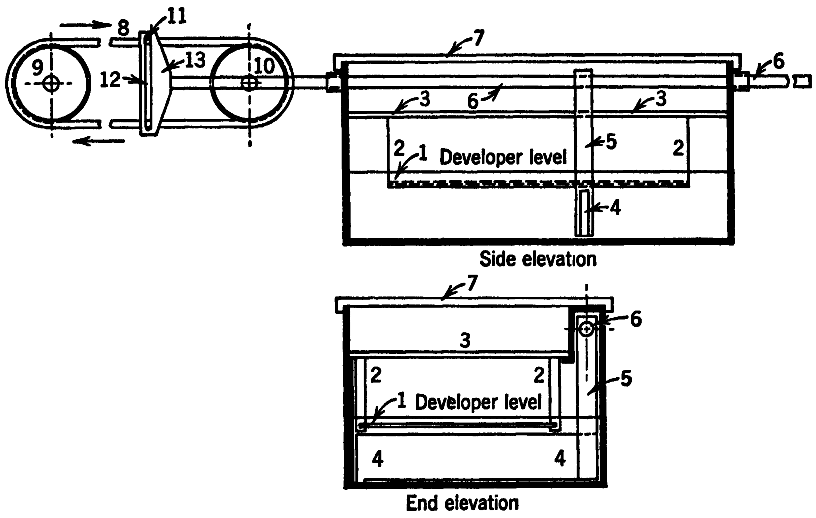


FIG. 6·16. Schuster developing tank.

6·23 Errors of spectrophotometry. Two classes of error may be distinguished, systematic and random. If two sets of repeat measurements be made with two instruments, and if the respective mean values of the two sets differ significantly, there is a systematic error between the two sets. If, on the other hand, the scatter about the mean (standard deviation) is greater for one set than for the other, we say that the random error is greater for the former set. Systematic errors affect the mean value of measurements; random errors affect the variance but not the mean. Random errors determine *reproducibility*. Random errors together with systematic errors determine *accuracy*. There follow likely sources of such errors for a typical split beam photometer, together with suggested means of testing for the error.

a. Systematic errors. To various causes may be attributed systematic errors of possible importance. Such errors depend very largely on the adjustment and cleanliness of the instrument. Theoretically they should be reducible to zero by correct calibration. It is always advisable to calibrate the scale of optical density over the whole wavelength range, since systematic error may vary with wavelength. Some of the kinds of such errors may be considered briefly.

1. Cells. Thickness should be measured with a micrometer gage. Leaks may be troublesome, especially when the solvent has a low surface tension. Fused-end cells are advisable for volatile solvents.

2. Calibration of variable stop. Incorrect values can be corrected only by the manufacturer. By varying thickness or concentration, one can test for constancy of molar extinction coefficient, ϵ,

$$\epsilon = \frac{1}{bc} \log \frac{I_0}{I}$$

An example of this test very thoroughly made on a photoelectric instrument is to be found in a paper by Vandenbelt, Forsyth, and Garrett (*41*). The principle is equally applicable to photographic methods. If ϵ is found to be constant, then one may assume that the instrument is free from systematic error. However, if ϵ is not constant, the cause may not necessarily lie with the calibration of the variable stop. All other possible causes of systematic error should be tested.

3. Inequality of the photometer beams. One aspect of this possibility is a simple geometrical asymmetry of the light paths. Complete symmetry is not obtainable for two beams issuing from the same side of a source of light if only for the reason that the aspect of the light source will be different for the two beams. This asymmetry may be small enough to be neglected as a source of error in a well-designed photometer.

Other causes of such inequality are the following: (*a*) unequal illumination owing to wrong position of the light source on the photometer or of the photometer on the spectrograph, or partial cutting off of one or both beams by the collimator of the spectrograph; (*b*) unequal transmission of light by the optics of the photometer or spectrograph due to unequal scatter or absorption by the quartz elements; and (*c*) partial deflection of one or both beams due to angular misalignment of the absorption cells, a possibility most likely with long cells (>2 cm.).

4. Matching. It has been shown (*18*) that preference of blackening is given to the lower of a pair of spectrograms being compared visually. It is often advisable to invert the plate and repeat matching where the density gradient is not steep.

A systematic error will occur through matching on the background spectrum rather than on lines. The error is greater than can be accounted for by the presence of stray light. No explanation of this effect is available. It is only of importance when visually matching line spectra.

There is a tendency, when matching visually, for the observer to be influenced by previous match points (see Matching, *b*1 below).

5. Wavelength calibration. It is advisable periodically to check wavelength calibration by means of a copper or mercury arc.

b. Random errors. Here also one or more of a number of different causes may contribute to error.

1. Matching. The error of visual matching contributes largely to the random error of measurement. A discussion of this point is given by Holiday and Irwin (*16*) and by Irwin (*21*), who found that the error amounted to about 1.5 per cent. This was reduced to about 0.6 per cent by matching with a photoelectric microphotometer. These results were obtained on lined spectra, which, as stated elsewhere, are not well suited for matching with a microphotometer. Much smaller error results when matching photoelectrically on continuous spectra, though there is little improvement in visual matching.

2. Reproducibility of blackening. The evidence on this point is scanty. Some unpublished work of Philpot (*33*) suggests that a value of about 0.005 to 0.007 unit of density is average for the standard deviation of the difference in density between any two points on a uniformly exposed plate and developed in a special agitating tank.

Dobson, Griffith, and Harrison (*11*), using a similar method of development, found variations in density suggesting a much larger error (0.02).

3. Wandering spark. This may be a source of error in a short-focus instrument of the Spekker type. The author finds that a much steadier spark is obtained by operating at 12,000 to 15,000 volts. The electrodes should be sharp.

4. Reading wavelength. Where the focal plane is curved and the photographic plate has to be bent around templates, considerable errors in wavelength reading may arise owing to the fact that the plate does not bow equally across its width. The oblique angle of incidence of the

light on the plate magnifies the error. Less trouble is encountered with spectrographs such as the Hilger E.498, which has a flat focal plane. In this case slight deviations from flatness of the glass or the gelatin emulsion may cause a similar error. The remedy is to use a control lined spectrum juxtaposed to the match pair and to use the lines as wavelength references. Plates can be made with the emulsion spread on plate-quality glass. The cost of such plates is prohibitively high for most work.

5. Dilution. In the test discussed by Holiday and Irwin (*16*) dilution was the second largest source of error. It seems that too great care cannot be taken in making up solutions.

6. Plotting and reading. In photographic spectrophotometry we find the wavelength at which a solution has a chosen optical density, whereas we usually wish to know the optical density at a chosen wavelength. Interpolation is therefore necessary. Errors may arise through plotting match points on a too contracted wavelength or density scale. Interpolation is most accurately made when the line through the match points makes an angle of 45° with the two ordinates (optical density and wavelength).

Various tests have been made to estimate the magnitude of the errors in spectrophotometric methods (*9*, *40*, *42*). It is difficult to express an over-all error by a single figure. The minimum for random error (reproducibility) for the photographic method is probably less than 2 per cent ($\sigma = 1.40$) when matching with photoelectric microphotometer and about 2 per cent when matching visually.

6·24 Some special applications of photographic registration to the characterization of absorption spectra. Some absorption spectra are extremely complex, such as those of vapors. Also some compounds, in solution, show absorption spectra of considerable degree of complexity. Such compounds are found among the salts of the rare earths and in the group of unsaturated polycyclic organic compounds. Spectrophotometry as such has been developed chiefly for the measurement of spectra of fairly simple character showing one or two bands with smooth contours. With the increasing application of the technique as an analytical method in organic chemistry and biochemistry, greater use is being made of subsidiary characters of absorption spectra as an aid to identification of chemical structure. The theoretical proposition that the absorption spectrum of a compound is specific for that compound is being explored to its limits. In this respect the photographic method offers possibilities which, in the opinion of the author, will always exceed those of the photoelectric method as a refined means for the recognition of fine structure in absorption spectra. Since this statement may be the subject of controversial opinion, it seems important to discuss the topic in some detail.

If a stepped optical wedge (Fig. 6·17 *A*) be viewed by transmitted light and a subjective estimate be made of the manner in which the density changes in a line at right angles to the divisions between the density strips, it will appear to most viewers that the density change does not take place in square edged steps but in the form diagrammatically illustrated in Fig. 6·17 *B*. The plot of a densitometer record actually made from the negative of Fig. 6·17 *A* is illustrated in Fig. 6·17 *C*. This contrast effect is due to the fact that subjective assessment of the density of a given small area depends on the densities of contiguous portions. This can be explained by reference to Fig. 6·18, in which one step is greatly enlarged. Zone 2 will appear lighter than zone 1 because zone 2 is next to the darker zone 3. Similarly zone 3 will appear darker than zone 4 because zone 3 is next to the lighter area zone 2.

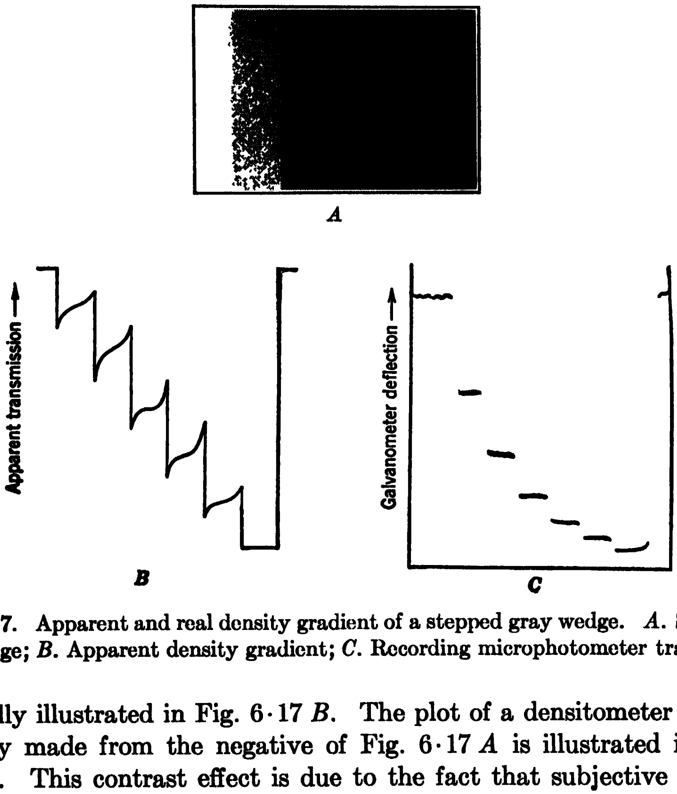


Fig. 6·17. Apparent and real density gradient of a stepped gray wedge. *A*. Stepped wedge; *B*. Apparent density gradient; *C*. Recording microphotometer trace.

That the effect is a subjective one is apparent from the densitometer record and from the fact that it disappears if the optical wedge is magnified until the grains of the image become apparent. The net effect is that the eye magnifies the density change between the steps. A complete explanation of the phenomenon has not yet been worked out. It must suffice to say that at least the following factors affect the definiteness of the phenomenon: (*a*) the intensity of illumination used for viewing; (*b*) the steepness of the change of density; (*c*) the difference in density; (*d*) the distance over which the change of density takes place.

This contrast effect, when applied to spectrograms, makes possible the detection of fine structure in absorption spectra with surprising sensitivity and precision. The band system of benzene in alcohol is clearly identifiable in the spectrogram of a solution containing 6 mg. of benzene per liter in a 10-cm. layer, although the optical density of such a solution is only 0.04 at the maximum of the strongest band.

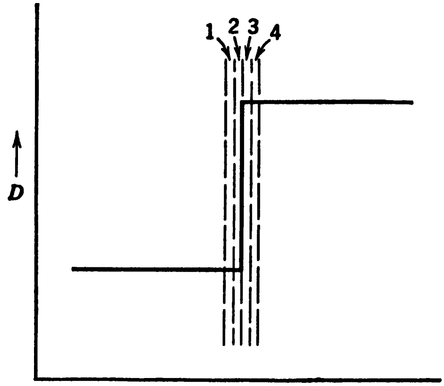


FIG. 6·18. Diagram to explain contrast effect on viewing stepped wedge.

Under well-chosen conditions, irregularities in the density distribution along the wavelength axis of a spectrogram that are undetectable by photoelectric densitometry are quite easily seen by inspection and the positions of the irregularities noted. For reasons of this kind crystallographic x-ray diffraction photographs are usually inspected and mapped by eye rather than by densitometer.

This phenomenon is utilized in a method of semiquantitative spectrophotometry developed by the writer (*14*), although without his knowledge the principle had been previously described in a paper by Brdička and Pavlick (*5*). In this method a photographic plate is raised in a vertical direction past the horizontal spectral image of the slit of a spectrograph (illuminated by a continuous source of light) at a rate which increases logarithmically as the distance traveled. Then the exposure of successive vertical portions of the plate will decrease logarithmically, and the density linearly with the distance the plate has moved (assuming the reciprocity law holds). If an absorbing medium be placed between the light source and the slit, then, assuming that the plate blackens equally at all wavelengths, a line drawn through all points having density equal to the density of the spectrogram at zero distance at a wavelength where there is no absorption will describe an absorption curve having extinction coefficient as linear ordinate. Reciprocity law failure and the unequal blackening of the plate at different wavelengths render the method unsuitable in its simple form to quantitative measurement of extinction coefficient. Its power of resolving fine structure in absorption spectra, as compared with what is possible with other methods, photographic or photoelectric, has led to its chief use.

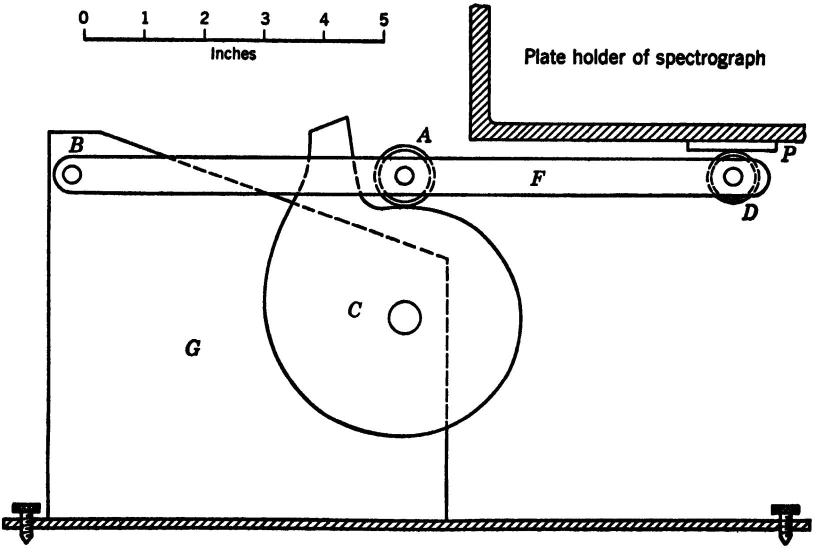


FIG. 6·19. Cam mechanism for raising plate at a logarithmically increasing rate.

By the introduction of a reference spectral strip exposed for the same time, through a control medium, as the least exposed portion of the graded strip, the second assumption is abolished and the method becomes reasonably quantitative. The method has the advantage of introducing no additional optics into the light path of a spectrograph, and it can be used with a vacuum spectrograph.

The motion of the plate is obtained by means of a logarithmically cut cam *C* (Fig. 6·19), which makes 1 revolution in about 6 minutes and is driven by a constant-speed motor. It bears on the tire of a ball bearing *A* mounted in a split arm *F*, pivoted at one end *B*. At the other end of the arm is another roller wheel D, which bears on a horizontal plate P attached to the plate holder. The pivot B takes all side thrust through a rigid back plate G, in which are mounted the bearing for B and that for the cam so that the wheel at P exerts only lift. The ratio of the lengths BD/AB determines the total lift and therefore the width over which the plate is exposed. In the early model the cam had a ratio of final to initial slope of 10:1. By allowing side thrust to be taken up by the pivot B, greater cam slopes were possible than in the original design and a ratio of 100:1 is now possible. Thus, a range of 2.0 in optical density of a solution can be covered.

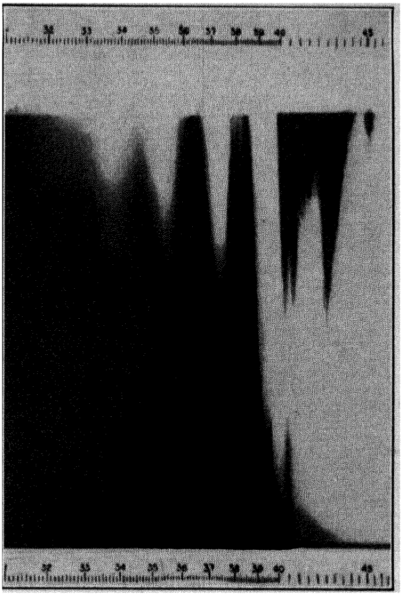


FIG. 6·20. Cam spectrogram of benzpyrene absorption spectrum, together with its fluorescence spectrum. The print is obtained by superimposing two spectrograms (fluorescence and absorption) so that the two wavelength scales coincide. The dark bands on the left represent absorption bands with absorbance index as ordinate. The dark bands on the right represent fluorescence bands with logarithm of intensity as ordinate. Absorption increases upwards; fluorescence intensity increases downwards.

Examples of spectrograms obtained by this method are shown in Figs. 6·20 and 6·21. The method is well adapted for taking fluorescence spectrograms.

The effect has been applied for two purposes: first, for more reliable enumeration of the band systems of spectra of high complexity, such as those of the polycyclic hydrocarbons (*17*), and, second, for the detection of traces of such compounds in the presence of other absorbing material showing more diffuse absorption (*18*).

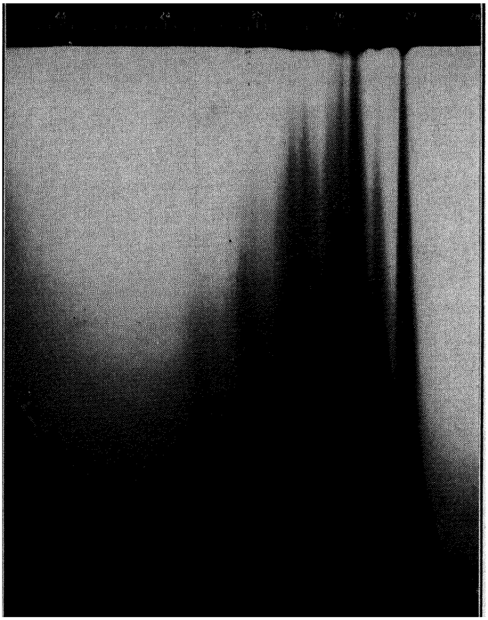


Fig. 6·21. Spectrogram of toluene in hexane; nineteen absorption bands can be distinguished on the original plate.

6·25 Photoelectric versus photographic methods. There is no doubt that photoelectric spectrophotometers are finding increasing favor and that in some laboratories photographic methods are considered too time-consuming, laborious, and inaccurate. This idea is in large measure true, but it seems worth while at this moment of change of fashion to consider without prejudice the relative merits of the two techniques.

It seems to the author that the outstanding advantage of the photoelectric spectrophotometer is that it enables one to select a wavelength at which to measure optical density. Among photographic methods this result is possible only with methods such as that of Philpot and Schuster (*32*). In all other photographic methods one has to arrive at the desired result by interpolation. In applied fields, especially where routine examinations are made of solutions with known absorption spectra but of differing concentration, the photoelectric method is of special advantage.

A certain caution is necessary, however, when working "blind" in this way. When using a photographic method, one always has at his disposal for observation a considerable portion of the absorption spectrum of the solution in question. It is often possible in this way to detect the presence of interfering absorption which would have passed unnoticed if one had been measuring at a single wavelength with a photoelectric instrument.

On the other hand, the measurement of complete absorption curves is probably as laborious and time consuming with nonrecording photoelectric as with photographic methods. In measuring the absorption spectra of new compounds it is important that as great a wavelength range of the absorption curve as possible be plotted for general purposes of reference. There is a tendency, when using the photoelectric method, to measure absorption only over a particular range that one is interested in for the problem in hand. Any part of the curve not measured at the time is not recoverable, whereas the photographic plate provides a permanent record even if one matches only a small portion of it at the time. That the photographic plate provides a permanent record is testified to by the thousands of plates which take up room in any spectrographic laboratory. The number of times one has occasion to refer to old plates is small, though the author has found the practice invaluable on several occasions in determining the times at which certain systematic error drifts have started to creep in, for example, wavelength calibration, focusing, zero correction of photometer, and decrease of short wavelength transmission by dirt on the optics. For all these data the photographic plate is a check and a permanent record. Unless adequate care is taken in devising routine checks of this sort for photoelectric spectrophotometers, many results may be vitiated.

Other things being equal, photoelectric methods should be more accurate than photographic procedures. The photocell is a more precise indicator of light flux than the photographic plate is an integrator of light energy. Moreover the number of energy transformations is fewer in photoelectric than in photographic measurements.

Photographic recording will continue to have particular value in special procedures, such as the detection of fine structure in absorption bands by the method described above (*14*). This is perhaps the only point at which the possibilities of the photoelectric method are more restricted than those of the photographic method. In photoelectric methods it is possible to arrange to expose the sample under test only to monochromatic radiant energy, whereas in all photographic methods the sample is exposed to the full range of radiant energy of the source. The former arrangement is of great advantage where determinations of optical density are to be made at one or two points in the absorption spectrum of a photosensitive compound. However, as the number of points measured increases, so does the total time of irradiation of the sample, until, when a whole absorption spectrum is measured, the total irradiation of the sample approaches and may exceed that necessary for the same measurement made photographically. If plenty of solution is available, the sample, of course, may be renewed at intervals. In the special case where as much as possible of the absorption curve of a photolabile compound is required, there is no doubt that the spectrophotometer of Philpot and Schuster shows advantages over other methods. A complete absorption curve may be obtained with this instrument with a total exposure of as little as 2½ seconds.

To summarize, it is the author's opinion that the photoelectric spectrophotometer is a more accurate and more convenient instrument than its photographic counterpart. For some special procedures photographic registration will continue to be superior.

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7· APPLICATIONS OF ULTRAVIOLET AND VISUAL SPECTROPHOTOMETRIC DATA

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A·INTRODUCTION

The present chapter is limited to spectrophotometric data. In selecting data to illustrate the applications of spectrophotometry, no consideration was given to the type of instrument with which the data were obtained. The only limitation was that transmittancy data should be available over a range of wavelengths.

The difference between a filter photometer and a spectrophotometer is not well defined. In general, filter photometers give transmittancy values at single wavelengths (actually the band passed by the filter), whereas spectrophotometers give transmittancy values as a function of wavelength. It is possible to take a series of readings with a filter photometer, using various "monochromatic" filters, and a plot of these transmittancy values versus wavelength will be a spectrophotometric curve. Such a curve, if the data are taken with narrow-band "monochromatic" filters, may well be better with reference to resolution than data taken with a spectrophotometer having a spectral band width of 35 mμ.

The present chapter is limited to data on transmittancy of liquid solutions. Some qualitative and quantitative analytical work has been done on the basis of reflectance measurements (*1*, *110*, *186*), but this has been in limited fields of application and is not of such general interest as transmittancy data. Analytical work on gases (*49*) is also excluded.

It is assumed that flawless transmittancy curves are available, and the problem is merely one of interpretation to obtain a maximum of useful information from the data. It is also assumed that the data are free from such instrumental errors as wavelength and transmittancy errors and have been obtained in solution cells of known length. The problems of eliminating instrumental errors have been discussed in Chapter 5. Where the measured transmittancy is that of a color developed by a chemical reaction with the component of unknown concentration, it is assumed that the chemical reaction has been carried out perfectly. The problems of eliminating chemical errors have been discussed in Chapter 1.

7·1 Symbols and definitions. Certain terms and abbreviations are used frequently throughout the chapter and are presented here rather than where they first occur. The Beer-Bouguer law terms are as follows.

$$T_s = 10^{-a_s bc}$$

$$k_s = a_s c = \frac{\log_{10} 1/T_s}{b} \tag{7·1}$$

$$A_s = \log_{10} \frac{1}{T_s}$$

where T_s = transmittancy, the ratio of the transmittance of the solution to that of the solvent in equal thickness.
a_s = absorbancy index in units of 1000 sq. cm. per gram.
c = concentration in units of grams per 1000 cc.*
b = cell thickness in centimeters.
k_s = absorbancy coefficient in units of reciprocal centimeters.
A_s = absorbancy.
mμ = millimicrons.

Additional symbols are used where needed and are defined in the text.

B·PRESENTATION OF DATA

7·2 Log absorbancy plots. Spectrophotometric plots with a log A_s (log log $1/T_s$) ordinate may be interpreted graphically with great speed and simplicity. If plots are made with this ordinate, the curve shape is a qualitative identification and the vertical height of the curve is a quantitative indication of the amount of material present. This will be apparent from the following discussion.

7·3 Independence of curve shape. Figure 7·1 illustrates that a uniform vertical displacement of curve results from a concentration change. Curve C of Fig. 7·1 is the absorption curve at concentration

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* One liter is not equal to 1000 cc., but the centimeter unit is used here for consistency in definition. Subsequently, the liter is used for illustrative calculations.

c of orange II (C.I. 151).* Although the ordinate of Fig. 7·1 is explicit in transmittancy, it is implicit in log A_s. Beer's law may be written in the form

$$\log (A_s)_1 = \log a_s + \log b + \log c \tag{7·2}$$

The concentration c and the cell thickness b are not functions of wavelength, and the shape of the curve will therefore be determined solely by log A_s, which is a function of wavelength. If a second solution is made up at a concentration of Fc, where F is a numerical factor, then the ordinates of the new plot (curve FC) will be

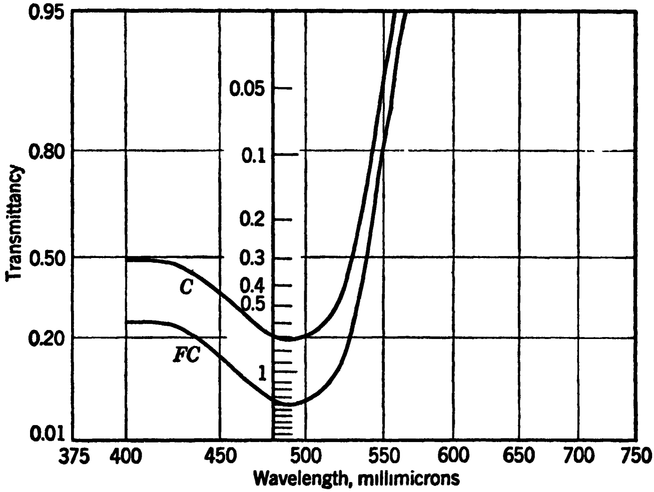

Fig. 7·1. Absorption spectra plotted in log absorbancy form.

C = curve of orange II (C.I. 151) at arbitrary concentration;
FC = curve of orange II at double the concentration of C.

Curves C and FC are vertically separated by a constant amount equal to log F.

$$\log (A_s)_2 = \log a_s + \log b + \log Fc$$

or

$$\log (A_s)_2 = \log a_s + \log b + \log c + \log F \tag{7·3}$$

Curve FC of Fig. 7·1 is the absorption curve of orange II, in which $F = 2$. It will be noted that equation (7·3) differs from equation (7·2) only by the term log F, which is a constant term independent of wavelength. As shown in Fig. 7·1, the plot of the material at a concentration Fc is vertically distant from the plot at concentration c by the amount log F.

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* *C.I.* is the abbreviation of *Colour Index*, a publication of the Society of Dyers and Colourists, which contains the chemical formulas of many dyes.

The independence of absorption-curve shapes when plotted on this ordinate is very important in qualitative analysis. If transparent plotting paper is used, it is possible to observe both curves at once by superimposing the sheets. If the sheet on which the curve of lower transmittancy is plotted is raised vertically by a linear distance equal to log F, the two curves will be coincident. Such a coincidence of curves is an indication of chemical identity. The vertical separation of the curves is very important in quantitative analysis, since this is log F. In comparing a curve of a standard with the curve of a sample of unknown concentration, F is the relative concentration.

A transparent logarithmic scale is convenient in the graphical analysis of curves of this type. The log cycle of the reference scale should be the same as the log cycle used for the ordinate of the spectrophotometric curves. Such scales can be conveniently produced photographically by making a positive film from an India ink drawing. This gives black numbers on a transparent photographic film. If this reference scale is placed on the log A_s spectrophotometric plot so that the reference scale falls along one wavelength and the 1 mark falls at 0.10 transmittancy, absorbancies may be read directly from the scale. The value of the reference scale at the intersection of a plotted spectrophotometric curve with the reference scale is the absorbancy of the solution at that wavelength.

Such a reference scale is illustrated in Fig. 7·1, where it is drawn at 480 mμ. It has been placed so that the 1 mark falls at 0.10 transmittancy and in this particular vertical orientation reads absorbancy directly. Thus, curve C has an absorbancy of 0.68 and curve FC an absorbancy of 1.36 at 480 mμ, as their intersection with the reference scale shows.

If the reference scale is placed along one wavelength, but in an arbitrary vertical position, the intersection of a spectrophotometric curve with it will not give a direct reading of absorbancy, but will give jA_s, where j is an arbitrary factor. This arbitrary factor may be evaluated by observing the reading on the reference scale at the 0.10 transmittancy intersection but is ordinarily not evaluated. This factor j is constant, provided the scale remains in one position. In Fig. 7·1, if the reference scale is placed at any wavelength, such as 450 mμ, and at an arbitrary vertical position, the intersection of curve C will give a reading of j log $1/(T_s)_1$, and the intersection of curve FC will give a reading of j log $1/(T_s)_2$. In accordance with Beer's law

$$\frac{[j \log 1/(T_s)_2]}{[j \log 1/(T_s)_1]} = \frac{a_s bcF}{a_s bc} = F \qquad (7\cdot4)$$

It will be noted that j cancels in equation (7·4). This means that the quotient of the two readings on the reference scale will give F, regardless of the vertical position of the scale. To read relative concentration, the transparent reference scale is ordinarily placed with 1 on the standard curve, so the relative concentration is read directly from the intersection of the curve of the sample.

7·4 Addition of curves. When two absorbing materials a and d are present together and have additive absorbancies, it follows that

$$\log \frac{1}{(T_s)_{a,d}} = \log \frac{1}{(T_s)_a} + \log \frac{1}{(T_s)_d} \qquad (7\cdot 5)$$

In qualitative analysis it is frequently desirable to be able to predict the curve of a mixture of components, or, having the curve of a mixture and one component, to be able to predict the curve which would result if the concentration of one of the two components were zero. These processes involve the addition and subtraction of curves and may be carried out conveniently in accordance with equation (7·5) by using the reference scale.

Figure 7·2 illustrates the principle of additive absorbancies and the use of the reference scale in the addition and subtraction of curves. Curves A and D are the curves of two components, and curve C is the curve of a mixture of components A and D present in the same concentration at which they occur in A and D. If the reference scale is placed along any one wavelength with its 1 mark falling at 0.10 transmittancy, the absorbancies of curves A, D, and C may be read off, and it will be seen that equation (7·5) is obeyed at each wavelength. It is possible to predict curve C, in accordance with equation (7·1), from the absorbancies of curves A and D.

When each term of equation (7·5) is multiplied by j, equation (7·6) is obtained:

$$j \log \frac{1}{(T_s)_{a,d}} = j \log \frac{1}{(T_s)_a} + j \log \frac{1}{(T_s)_d} \qquad (7\cdot 6)$$

Equation (7·6) signifies that regardless of the vertical position of the reference scale, provided it is held fixed for one addition, the values read at the intersections of curves A and D, added, will give the value at the intersection of curve C. It has been found convenient in predicting the curve of a mixture to proceed as illustrated by scale G of Fig. 7·2. The scale is placed so that the 1 mark falls on one curve, the intersection of the other curve is read on the scale, the addition (1 plus the intersection reading) is easily carried out mentally, and a mark is made on the plot at the sum on the reference scale. Repeating this procedure at various wavelengths gives enough points to draw in a predicted curve *C*.

The process of predicting curve *A*, given curves *D* and *C*, is carried out in a similar fashion and is illustrated by reference scale *E* of Fig. 7·2. It has been found convenient to place the 1 of the reference scale on the curve of the mix, to read the value at the intersection of curve *D* and the reference scale, to perform the subtraction mentally, and to plot a point on the sheet at the difference on the reference scale. A number of such points, determined at various wavelengths, define the curve.

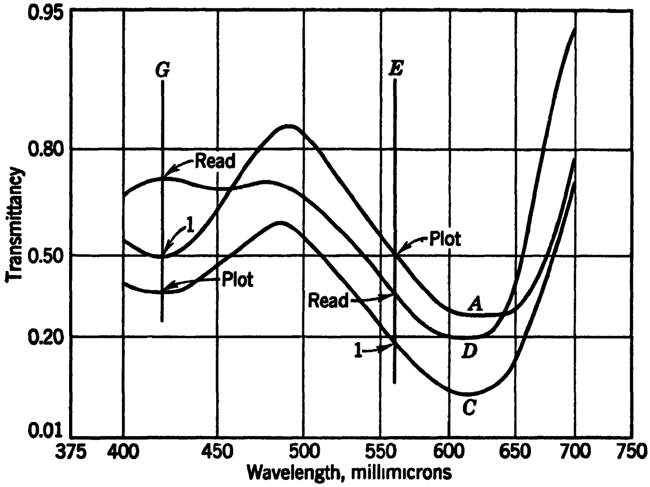

Fig. 7·2. Graphical addition and subtraction of curves.
A = alizarin cyanine green (C.I. 1078);
D = naphthol blue black (C.I. 246);
C = mixture of *A* and *D* at the same concentrations but in one solution.

It will be noted that for comparing curve shapes and predicting curves of components and mixes, which are useful in qualitative analysis, and in determining *F*, which is useful in quantitative analysis, no readings were taken from the ordinates of the spectrophotometric plots. In order to use the logarithmic reference scale for these purposes, it is necessary that the plots be implicit in log A_s, but no requirement is placed on the explicit ordinate. It has been found convenient to label the ordinate in transmittancy. This does not give a linear scale, but it does mean that transmittancy data, obtained from any instrument, may be plotted by direct reference to the explicit ordinate, and the resulting curve will have all the useful properties of the log A_s plot.

Absorbancy index data may be transferred to this plot by placing the 1 of the reference scale at 10 per cent transmittancy and making a point on the plot where the value of a_s falls on the reference scale. Such a set of points, at different wavelengths, comprises the spectrophotometric curve corresponding to data obtained in a 1-cm. cell with a concentration of 1.00 g./l.

Molar absorbancy index data (in units of 1000 cm.^{2} per mol) may be transferred to this plot in the same manner as the a_s data, except that, instead of 1, the molecular weight of the compound read on the reference scale is placed at 10 per cent.

Absorbancy index data may be read from this type of plot by placing the reference scale so that the reciprocal of the concentration in grams per liter falls at 10 per cent transmittancy. The intersection of the curve with the reference scale then gives the absorbancy index in grams per 1000 cm.^{2}

Molar absorbancy index data may be read from this type of plot by placing the reference scale so that the quotient of the molecular weight by the concentration in grams per liter is read on the reference scale at its intersection with 10 per cent transmittancy, and the molar absorbancy index in mol per 1000 cm.^{2} is then read from the intersection of the plotted curve with the reference scale.

The spectrophotometric data throughout this chapter are presented on plots which are explicit in transmittancy and implicit in log A_s. For laboratory work it is recommended that plots be made on transparent paper and that a logarithmic reference scale be available. Logarithmic cycles of 7.5 and 37.5 cm. have been found useful.

7·5 Specification of data. The presentation of quantitative spectrophotometric data is an important subject which has received the attention of a few individuals (*134*, *135*) but which will probably require the attention of some sort of an intersociety committee before the published data in the many and varied fields of application are made uniform. Uniformity will be useful because, when full cognizance is taken of sources of variation, the data obtained in different laboratories will be found to check well (*50*, *213*). This will mean that less time will be required for duplication of work in various laboratories and more time will be available for the advancement of the science.

Before such unification can be accomplished, it is important that each author give complete details regarding his data. The compound measured should be adequately described so that its purity and likely impurities are known. The instrumental methods should be fully described so that such important factors as spectral resolution are indicated. The solution conditions should be fully described. Recommendations for describing the sample and instrument are outside the scope of this chapter. So far as solution conditions are concerned, it is recommended that all the solution factors discussed in Sections 7·8 to 7·23 be evaluated and a statement made as to the means of controlling each of them.

7·6 Available data. An available compilation of spectrophotometric data obtained in different laboratories is the *Catalog of Ultraviolet Spectrograms* of the American Petroleum Institute Research Project 44 at the National Bureau of Standards (1946). This compilation is not perfect. In some cases the instrument used for analysis is not stated. In others the slit width is stated in millimeters, and considerable detailed knowledge of the instrument is required in order to determine the spectral resolution in terms of slit width and slit shape. In still other cases such an important solution condition as kind of solvent is not specified.

International Critical Tables contain both absorption data themselves and references to literature containing absorption data. These are found for inorganic materials in the sections by Emma P. Carr and Mary L. Sherrill, Vol. V, p. 326 (1929); and J. Becquerel and J. Rossignol, Vol. V, p. 268 (1929). Data on organic materials are found in sections by Emma P. Carr and Mary L. Sherrill, Vol. V, p. 326 (1929); and V. Henri, Vol. V, p. 359 (1929). Data on dyes are found in the section by W. C. Holmes, Vol. VII, p. 173 (1930). Later data will be found in Sections 1 and 2 of the *Annual Tables of Physical Constants and Numerical Data,* edited by N. Thon and published in recent years under the auspices of the American Committee on Annual Tables, National Research Council, publication office Frick Chemical Laboratory, Princeton, N. J.

Foreign tabulations of data include F. Ellinger, *Tabulae Biologicae,* Vol. XII (1937); V. Henri and L. Bruninghaus, *Tables annuelles de constantes et donnees numeriques, donnees numeriques de spectroscopie,* Vols. I to XII (to 1936); and Landolt-Börnstein, *Physikalische-chemische Tabellen,* edited by W. A. Roth and K. Scheel (to 1934). In addition, literature references to absorption spectra will be found in Beilstein, *Handbuch der organischen Chemie,* 4th edition (to 1941), and in Gmelin, *Handbuch der anorganischen Chemie,* 8th edition (to 1941).

Brode has critically evaluated most of the available collections of data (*32*).

As noted in Chapter 2, the reader must be on the alert to avoid confusion because of the use of different definitions and symbols in various publications.

C·SOLUTION VARIABLES

Materials absorb radiant energy in the ultraviolet and visual ranges, and therefore have spectrophotometric curves, because of electron transfers from lower to higher energy states. In salts of the rare earths these electron transfers occur within the atom and are remarkably unaffected by external conditions, but for the majority of materials the external solution conditions affect the transfer and alter the observed absorption curves. A great many applications of spectrophotometric data are based upon this fact. Measurements of absorption curves are used to evaluate and follow changes in various solution conditions. Thus measurements of absorption curves of acid-base indicators are made, not to determine the characteristics of the indicator, but to determine the hydrogen-ion activity of the solution.

Solution variables are also of importance from another viewpoint; unless they are controlled, qualitative and quantitative analyses are likely to be in error. As an example, consider the determination of the concentration of phenolphthalein in water solution. If the standard is made up at a *p*H of 9, it will have a red color. A solution of unknown concentration might be made up at a *p*H of 3, since *p*H is not controlled, and, if so, will have no color, regardless of the concentration of the indicator dye. A quantitative analysis in the visual range will give the incorrect value zero for the unknown.

Because of the great importance of solution variables, this entire section is devoted to them.

7·7 Factors of importance. Each factor giving rise to an effect is discussed only briefly, the purpose being to indicate its importance rather than to present a review of all the work that has been done in connection with it. Each effect should be considered from its dual aspect; by measuring the material the effect may be studied, and by controlling the effect the material may be determined.

7·8 Solvent. Kundt noticed in 1878 (*116*) that the properties of the solvent affect the absorption curve of a material in solution. He believed that the absorption band is shifted toward the red end of the spectrum with an increase in the refractive index of the solution. Brode showed (*30*) that this was not the case. He studied the effect of 29 organic solvents on the absorption curve of benzeneazophenol and concluded that there was no relation between the lateral position of the absorption curve and the refractive index of the solvent. Regardless of the cause, absorption curves of the same material may differ widely in different solvents. Such an effect is illustrated by curves *C* and *D* of Fig. 7·3. Curves *C* and *D* are very much alike in their general shape, but curve *D* is found at slightly longer wavelengths. Changing the solvent from 1,2-dichlorobenzene to pyridine has the effect of moving the absorption curve laterally to longer wavelengths. When any factor causes a shift to longer wavelengths, it is said to have a *bathochromic* effect. Conversely, a change in solvent from pyridine to 1,2-dichlorobenzene has the effect of shifting the absorption curve without much change in shape toward shorter wavelengths, and such an effect is called a *hypsochromic* effect. Two other terms, less frequently used, are *hyperchromic* effect, in which the absorption curve is changed toward a region of lesser transmittancy without much change in curve shape, and *hypochromic* effect, in which the absorption curve is moved toward a region of greater transmittancy without much change in curve shape, a requirement for both cases being that the concentration remain the same.

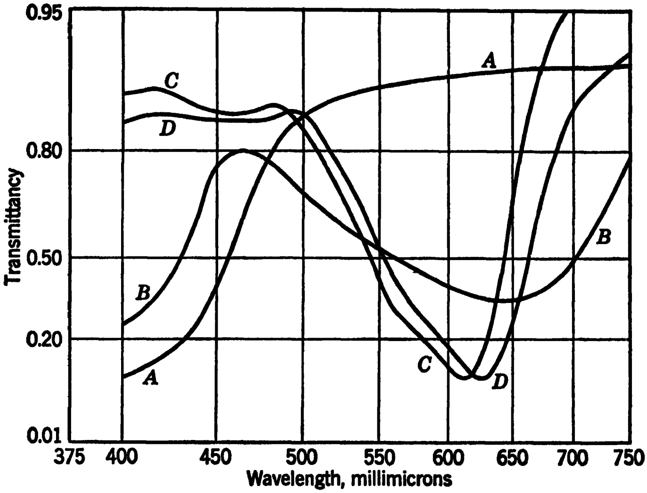

FIG. 7·3. Effect of solvent on absorption spectra of indigo MLB/2B (C.I. 1183).

A = water with sodium hydrosulfite;
B = sulfuric acid;
C = 1,2-dichlorobenzene;
D = pyridine.

Concentrations arbitrary.

Greater changes in absorption curves will occur when the solvent reacts with the material. Chemical reactions may occur with solvents such as water which are ordinarily thought of as being inert. For instance, it has been shown that formaldehyde in hexane at −70°C. has an ultraviolet absorption curve corresponding to that observed for the unimolecular gaseous condition, but in water it appears to be completely hydrated (*85*). Sometimes materials are dissolved in strong acids or bases, or other reactive solvents, with the intention of bringing about a chemical change which will assist in the qualitative identification of the material. The use of this method in dye identification has been described (*1*, *186*). Curves *A* and *B* of Fig. 7·3 illustrate the effect of reactive solvents.

For mixed solvents, the change in the absorption curve is not always linear with the change in the proportion of the two solvents. Brode noted that, if a strongly polar solvent (alcohol) is mixed with a weakly polar solvent (benzene) and benzeneazophenol is dissolved in the mixed solvent, the absorption band assumes the position it would have in 100 per cent alcohol. This is true even if the amount of alcohol present in the benzene is as little as 1 per cent. Similar results were obtained with iodine in carbon tetrachloride (nonpolar) and ethanol (polar) (*28*). The nonadditivity of solvent effect is illustrated by Fig. 7·4. It will be observed that the difference in curves *A* and *B* at 600 mμ, which is brought about by the addition of 1 per cent ethanol to water, is much greater than would be predicted on the basis of the difference between curves *A* and *C*, which is caused by 30 per cent ethanol.

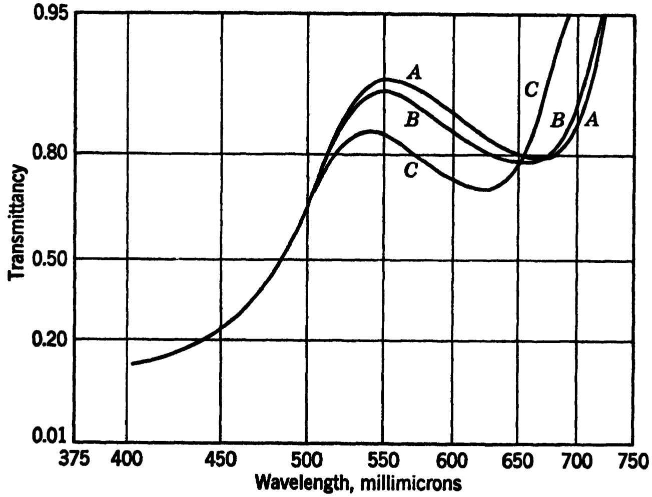

FIG. 7·4. Effect of solvent on absorption spectra of a mixture of 75 per cent chrysophenine (C.I. 365) and 25 per cent diamine sky blue FF (C.I. 518).

A = 100 per cent water as solvent;
B = 99 per cent water and 1 per cent ethanol;
C = 70 per cent water and 30 per cent ethanol.

The absorption curve of quinizarine dissolved in hexane shows some sharp peaks which disappear as the solvent is changed by the addition of nitrobenzene. This loss of fine structure has been used as an indication of the polarity of the nitrobenzene (*206*).

7·9 Hydrogen-ion activity. Perhaps the first report of a change in color of a solution caused by a change in hydrogen-ion activity was given in 1660 by Boyle (*21*), who noted the color changes when solutions of several naturally occurring organic materials were treated with various acids and bases. The effect of *p*H on the absorption curves of solutions and the interpretation of these data in terms of ionization constants have been studied extensively over a period of years, notably

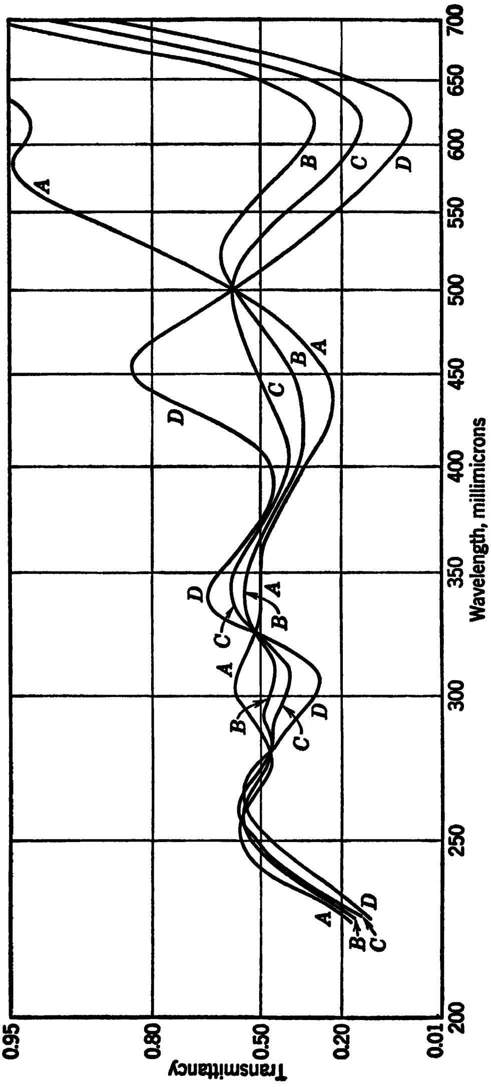

FIG. 7·5. Absorption spectra of bromothymol blue at various hydrogen-ion activities.

A = pH 5.45;
B = pH 6.95;
C = pH 7.50;
D = pH 11.60.

Solvent = water; a_s = 60.5 at 615 mμ for D.

by Acree and coworkers (*2*, *170*). The application of a spectrophotometer to the determination of the *p*H of a solution by measuring the relative amounts of the acid and basic form of an indicator dye dissolved in the solution has been pointed out (*27*).

If it is desired to measure the concentration of a material present in a solution which is subject to changes in absorption-curve shape with changes in *p*H, a buffer may be added. Addition of buffers to solutions of slightly soluble compounds such as some dyes may cause polymerization and precipitation, a phenomenon known in the dyestuff industry as "salting out."

The effect of *p*H on a sensitive material is illustrated in Fig. 7·5, where curves are shown of bromothymol blue at various hydrogen-ion activities. It will be noted that there are certain wavelengths, 279, 324, and 501 mμ, where all the curves cross at a common point. Such a point is known as an isosbestic point. The term *isosbestic point* is applied to any wavelength where the absorbancy indexes are the same for two materials which are interconvertible, as in hydrogen-ion activity, redox potential, or tautomeric equilibria.

7·10 Concentration. Beer announced in 1852 (*12*) his theory of the effect of concentration on transmittancy, which states in effect that absorbancy indexes are independent of concentration. Ever since then exceptions to his law have been reported. An example of variation of absorption-curve shape with concentration is shown in Fig. 7·6. Because the product of cell length and concentration is the same for the conditions of measurement of both curves *A* and *B* of Fig. 7·6, the curves would be superimposed if the absorbancy indexes were constant. They obviously are not. Probably, however, the truth of the matter is that Beer's law holds strictly for any one molecular or ionic species, and deviations from the law indicate the presence of at least two molecular species in equilibrium, the equilibrium being subject to variation with concentration. Thus intermediate concentrations of formyl violet S4B all possess the same absorbancy index at 558 mμ, this being an isosbestic point. This is a qualitative indication that two, and only two, molecular species of dye are present in all the solutions.

Studies of Beer's law deviations, and what the deviations signify in terms of the material in solution, have been the subject of much work, notably in the study of dyes (*68*, *96*, *165*, *182*). If it is desired to evaluate the concentration of a material in solution and not introduce an error because of Beer's law failure, it is possible to limit the measurements to a narrow range of concentrations, work at an isosbestic point, change to another solvent, or make suitable corrections. A detailed treatment of these points will be found in Division E.

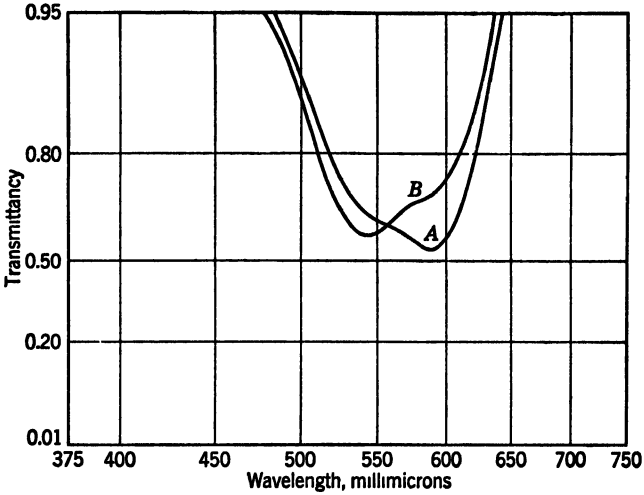

Fig. 7·6. Effect of concentration on absorption spectrum of formyl violet S4B' (C.I. 698).

A = 0.4 mg./l. in a 10-cm. cell;
B = 20.0 mg./l. in a 0.2-cm. cell.

Solvent = water; a_s = 68 for A at 588 mμ.

7·11 Temperature. Sometimes the shape of the absorption curve of a solution prepared for spectrophotometric measurement varies with temperature (*114*). For instance, when an absorption curve is measured at 20°C. one shape will result, when the absorption curve is measured at 40°C. a different shape will result, and when the solution is cooled to 20°C. the shape obtained will be identical to the first. This is a reversible temperature effect and is well illustrated by the dye eriochrome verdon A (C.I. 292). Figure 7·7 shows the reversible change in absorption-curve shape which this dye undergoes when heated in water at a *p*H of 9 over a temperature range of 25°C.

Another reversible temperature effect, which is less commonly encountered in most work, involves a large change in temperature. Thus the absorption spectrum of dodecapentaenic acid in an ether-alcohol solution shows much better resolution of the absorption band at −196°C. than at +20°C. (*123*).

Another effect may be an irreversible change in absorption-curve shape when the solution is heated, but this is discussed below as a solution heating effect.

Thionine and methylene blue illustrate a temperature effect which may be ascribed to polymerization (*165*). Thionine at a concentration of 2.5×10^{-3} M shows a considerable change in the shape of the absorption curve over the temperature range of 10 to 70°C. The change induced by higher temperature is essentially an increase in the absorbancy coefficient of the absorption band at 597 mμ at the expense of the band at 557 mμ. When the concentration is reduced to 2.5×10^{-5} *M*, a similar change in curve shape occurs, the 597-mμ absorption band increasing greatly. Thus increasing temperature results in the same change as increasing dilution. Furthermore, the more dilute solution shows practically no temperature effect, the absorption curve retaining its shape over a range of temperatures. Apparently the increased temperature of the concentrated solution results in a change toward the molecular species existing in dilute solution. The conclusion of this study of thionine is that the absorption at 597 mμ is accounted for by a monomeric ionic form, and the absorption at 557 mμ is accounted for by a dimeric ionic form, increasing temperature and increasing dilution both favoring the monomeric form.

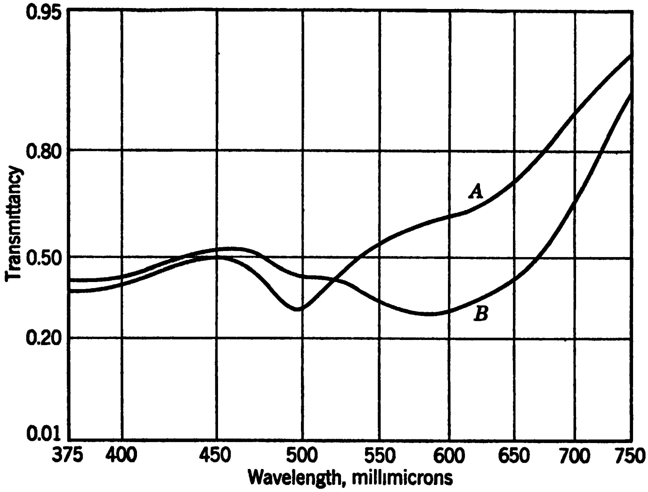

FIG. 7·7. Effect of temperature on absorption spectrum of eriochrome verdon A (C.I. 292).

A = 25°C.;
B = 50°C.

Solvent = water; a_s = 12 at 590 mμ for B.

Similarly, in the case of rhodamine B and other fluorescent dyes, it has been found that identical absorption-curve shapes are induced by lowering the concentration or by raising the temperature (*182*).

A change in the shape of the absorption curve of eriochrome verdon A from that of curve *A* to curve *B*, shown in Fig. 7·7, may be brought about not only by raising the temperature but also by increasing the pH of the solution. This suggests that the temperature effect is related to a change in the dissociation constant with temperature. This view is substantiated by the fact that tests carried out at pH values far removed from the pK value (which is about 9.5) show that the temperature effect is greatly reduced.

The effect of temperature on acid-base indicators has been studied by Clark (*45*), who has found, for instance, that the same color of thymol blue is observed at a pH of 9.4 and 18°C. or at a pH of 9.0 and 70°C.

7·12 Heating of solution. The temperature at which the dissolution of a material is effected may have an influence upon the absorption curve obtained. This irreversible temperature effect is illustrated in Fig. 7·8 for the dye diamine violet N. As Holmes (*95*) showed, this dye, when dissolved in water at room temperature, gives a characteristic absorption curve which is stable over periods of 24 hours, provided the temperature is kept below 25°C. When the solution is heated to 85°C., a change takes place in the absorption curve, as illustrated in Fig. 7·8. Upon being cooled to its original temperature, the dye does not regain its original absorption characteristics but maintains the color it assumed when first heated.

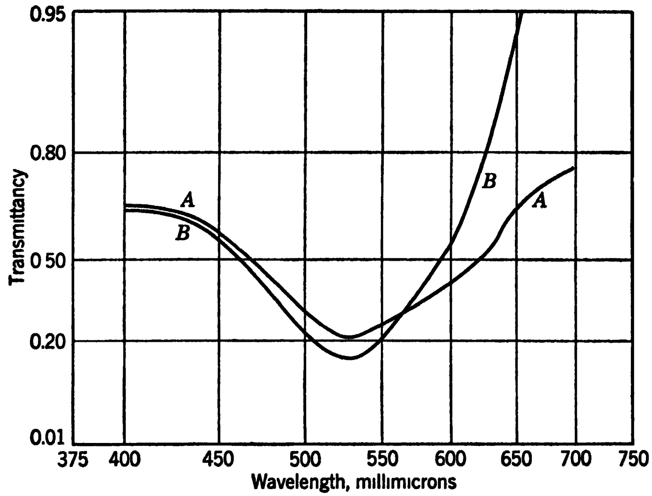

FIG. 7·8. Effect of solution heating on absorption spectrum of diamine violet N (C.I. 394).

A = dye dissolved at 25°C.;
B = dye dissolved at 85°C.

Solvent = water; a_s = 20 at 525 mμ for B.

In order to obtain the complete absorption curve of some compounds on the log A_s plot, it is necessary to prepare solutions at two or even three concentrations. This is ordinarily accomplished by dissolving a large amount of material to get a concentrated solution and then diluting it. Dissolving a large amount of material has the additional advantage with samples of poor homogeneity of giving better sampling, and therefore this procedure may be used even when it is not necessary from the viewpoint of getting absorption data over a greater range of wavelengths. It is sometimes found that, although the initial solution has been heated, the dilute solution may show a heating effect of this irreversible type. Some workers have advocated letting dilute solutions stand for 24 hours before measurement in order to insure reproducibility of measurement of such solutions (*120*). Usually the attainment of stability can be hastened by heating the dilute solution.

7·13 Interfering ions. The formation of complexes is perhaps the most important solution effect. It is the basis of much of the great quantity of analytical colorimetry in which a color is developed (*180*).

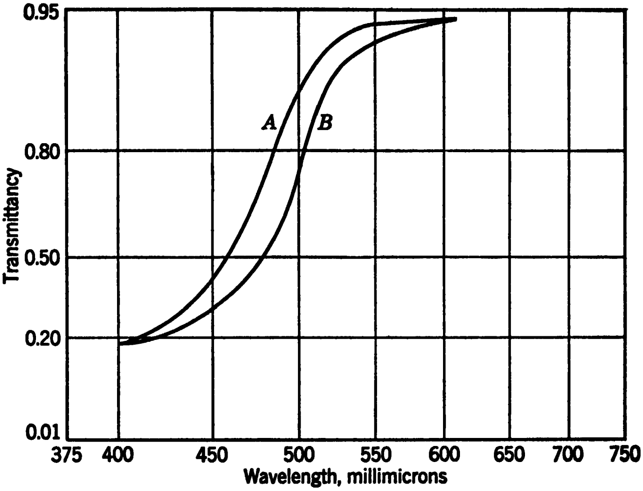

FIG. 7·9. Effect of interfering ions on absorption spectrum; complex formation of benzo fast yellow 5GL with copper.

A = distilled water;
B = with 0.1 mg./l. of Cu^{++} ion added.

a_s = 25 at 400 mμ; see Stearns (*184*).

Failure to recognize it may be a major source of error in all analytical colorimetry. Figure 7·9 shows the absorption curve of benzo fast yellow 5GL in distilled water (curve *A*) and the large change which is caused by 0.1 mg./l. of bivalent copper ion (curve *B*). Failure to control the amount of copper present in the water will lead to analytical errors when working with this dye. To avoid such effects, extreme purity of solvents and reagents is in general desirable. Chapter 1 discusses this type of "solution effect" in detail.

7·14 Interfering ions. Ionic strength. It is noted experimentally that for some colored materials a different absorption curve is obtained, depending upon the concentration of other materials present, such as sodium chloride. The sodium chloride itself contributes no color, and it probably does not form a complex with the material; but its presence affects the colored material and thereby introduces a color change. This effect is illustrated by Fig. 7·10. Curve *A* is the absorption curve of stilbene yellow 2G (C.I. 622) as it would normally be measured, prepared in distilled water and having only a low concentration of weak buffering salts added. Curve *B* is the absorption curve resulting from making the dye up in a solution of 1 per cent sodium chloride but with other conditions alike. It is evident that, if the salt content of the water used in obtaining curve *B* were not recognized, erroneous conclusions would be drawn from the curve as regards identity and quantity of component present.

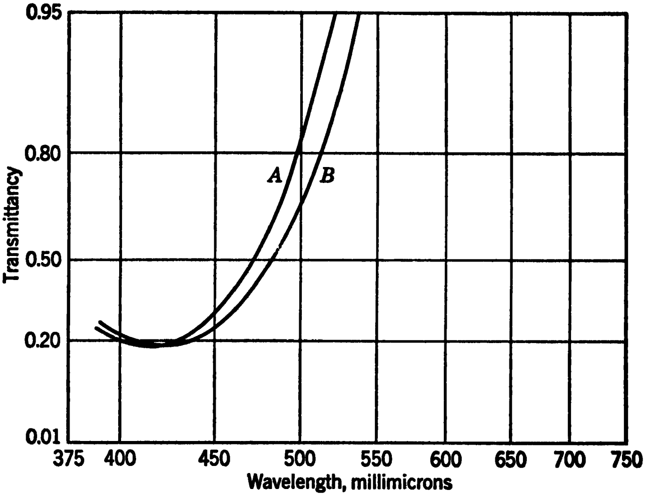

FIG. 7·10. Effect of ionic strength on absorption spectrum of stilbene yellow 2G (C.I. 622).

A = distilled water;
B = 1 per cent NaCl solution.

Solvent = water; a_s = 25 at 415 mμ for *A*.

The effect of high ionic strength on the color of material has been the subject of considerable study (*121, 124, 133, 165*).

7·15 Irradiation. It was noted by Marckwald (*130*) in 1899 that salts of quinoline and some tetrachloroketonaphthalenes exposed to sunlight change in shade and revert to normal color upon dark storage. In the spectrophotometry of solutions it may be found that the absorption curve of the material will change upon exposure of the solution to light, and after storage in the dark the curve shape will return to the original. This is a reversible light effect and is differentiated from light fading, which is discussed later, by its reversibility. The frequency of occurrence of this effect among dyes has been found to be about 9 per cent (*185*). The effect is of varying magnitude, and the dark storage recovery time varies from 30 seconds to 3 days at room temperature.

In spectrophotometers which monochromate and then irradiate the solution for measurement, the intensity of light striking the sample is ordinarily so low that irradiation is not a factor. With instruments in which the sample is irradiated and the light subsequently monochromated, the intensity of light striking the sample is often high enough to introduce an irradiation effect (see Chapter 5).

The irradiation effect may be very strikingly illustrated with a solution of $10^{-5}\,M$ of methylene blue and $0.02\,M$ of ferrous ion at pH 3. A strongly blue solution in a test tube is completely decolorized by the irradiation from a 100-watt tungsten lamp when held 10 inches away for 6 seconds, and dark storage brings about a complete return of color in about 1 minute. A theory which has been proposed (*164*) to explain this phenomenon is that an oxidation-reduction reaction takes place, the light displacing the equilibrium toward reduction of the dye and oxidation of the iron.

The above system exhibits an interesting photogalvanic effect which further substantiates the oxidation-reduction hypothesis. There is a tendency for the leucomethylene blue to deposit an electron on a platinum electrode, thereby becoming oxidized to the blue form. The ferric ion picks up the electron from the electrode. The tendency to deposit electrons on the electrode varies, being greater in an irradiated solution (where the concentration of leucomethylene blue is greater). If a single solution as above is placed with one half irradiated by light and one half in the dark, and if two electrodes are placed in the solution, one in the lighted portion and one in the dark portion, closing an external circuit will permit electric current to flow from one electrode to the other. There is thus a conversion of light to electricity, if the solution is mixed, which may continue indefinitely except for extraneous fading of the dye.

Another compound which has received much attention as regards oxidation-reduction equilibrium changes with light is chlorophyll (*127*, *163*).

Figure 7·11 illustrates a change in curve shape which may occur upon irradiation. This particular dye has a very slow recovery. About 3 days' dark storage at room temperature is required for the curve to return to the shape illustrated by curve *A* after irradiation.

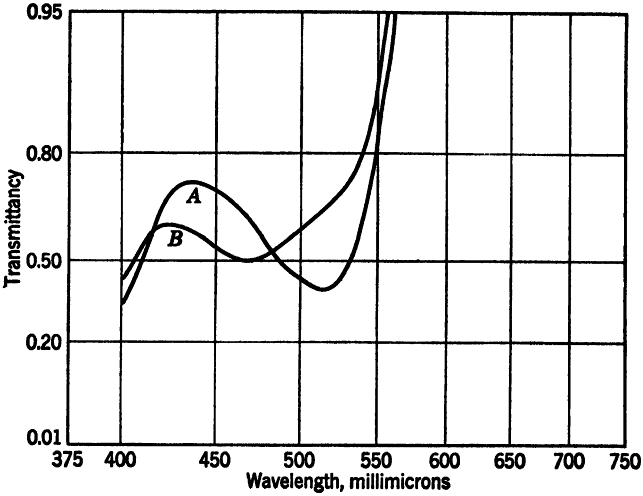

FIG. 7·11. Effect of irradiation on absorption spectrum of thioindigo orange R (C.I. 1217).

A = curve after sufficient dark storage;

B = curve after irradiation by 50-watt tungsten lamp for 1 min., 6 in. distant.

Solvent = pyridine; a_s = 33 for *B* at 515 mμ; see Stearns (*185*).

7·16 Redox potential. The fact that the color of a solution may be affected by dissolved oxygen has been known at least since 1843, because at that time von Helmholtz (*200*) used a dye to determine the degree of oxygen exhaustion from a solution. This effect is taken advantage of in oxidation-reduction titrations where a colored indicator is used (*207*).

A color change due to oxidation has been reported as interfering with spectrophotometric measurements. Boulton and Morton (*20*) found this effect troublesome when working with the easily oxidized leuco solutions of vat dyes. Holmes (*97*) found that various quinoneimide dyes such as methylene blue are so susceptible to alkaline oxidation that their conversion to the dye base may be attended by the loss of an alkyl substituent group.

On the other hand, this effect has been used to advantage in the analysis of ascorbic acid. When ascorbic acid is made up in water solution at a concentration of 0.002 *M*, it has an absorption spectrum shown by curve *A* of Fig. 7·12. This compound is rather easily oxidized by air to the nonabsorbing dehydroascorbic acid. Consequently, its absorption decreases upon standing. When air is bubbled through a solution of ascorbic acid, the apparent loss in concentration is shown by curves *B* and *C* of Fig. 7·12 (*148*). This oxidation is accelerated by the cupric ion and retarded by 0.002 *M* potassium cyanide.

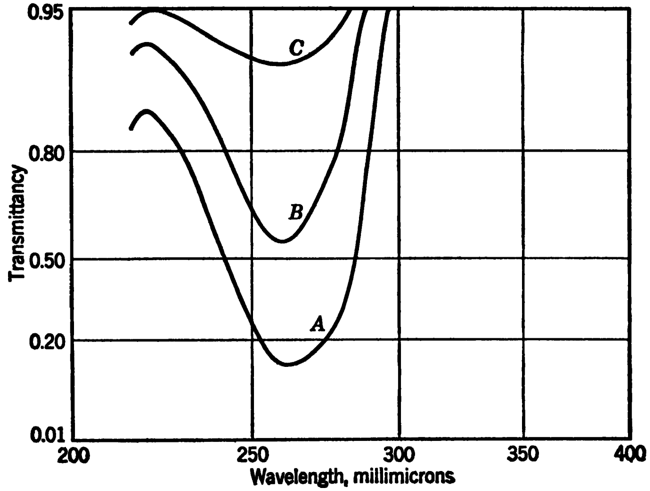

FIG. 7·12. Effect on absorption spectrum of bubbling air through a solution of ascorbic acid (vitamin C) (redox potential effect).

A = initial solution;
B = after 1 hour;
C = after 5 hours.

Solvent = water; a_s = 35.5 for *A* at 265 mμ; see Mohler and Lohr (*148*).

7·17 Dissolution aids. In order to get a true solution of a substance it is helpful to raise the solvent to a high temperature, stir vigorously, and allow ample time. In some cases this procedure requires an exorbitant effort or has a destructive influence on the substance, and then resort may be had to a chemical aid to effect dissolution. The chemicals used may vary widely, but the effect on the absorption curve should always be determined.

Many dyes are more easily brought into solution with the assistance of a nonionic surface-active agent, such as a reaction product of ethylene oxide and a long-chain carbon compound (*4*, *194*). This agent usually affects the absorption curve. For instance, Fig. 7·13 shows a water solution of diamine green B (C.I. 593) in pure water, curve *A*, and in water to which 1 per cent of nonionic surface-active agent has been added. It might be argued that this should be classed as a solvent effect, and indeed the addition of 1 per cent of a surface-active agent has much the same effect as adding a large amount of pyridine (*156*), but in any event the effect must be taken into consideration.

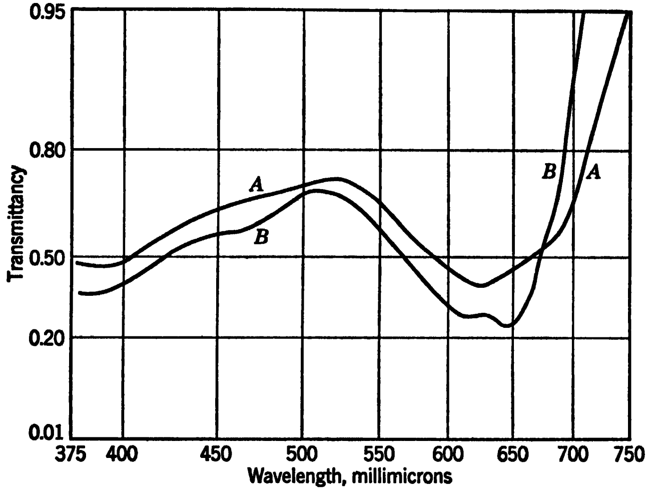

FIG. 7·13. Effect of dissolving aids on absorption spectrum of diamine green B (C.I. 593).

A = distilled water;
B = 1 per cent solution of a nonionic surface-active agent.

$a_s = 25$ at 645 mμ for *B*.

Pinacyanol chloride, a cyanine dye, is more soluble in a soap solution than in water. When potassium myristate is added to water in a high enough concentration, the soap forms micelles. The dye selectively dissolves in these micelles. The large change in the spectral absorption curve that is observed when the dye changes from a dimeric form in water to a monomeric form in the micelles has been used as an indication of the concentration of soap at which micelles are first formed (*52*, *53*, *54*, *55*, *131*).

7·18 Component interaction. The application of the principles of additive absorbancies to mixtures of two components, by means of which the absorption curve of a mix can be predicted from the absorption curves of the components, assumes that each component acts independently of the other. In many cases experiment has shown this to be true. On the other hand, as illustrated by the data of Fig. 7·14, two or more components in the same solution may affect each other,

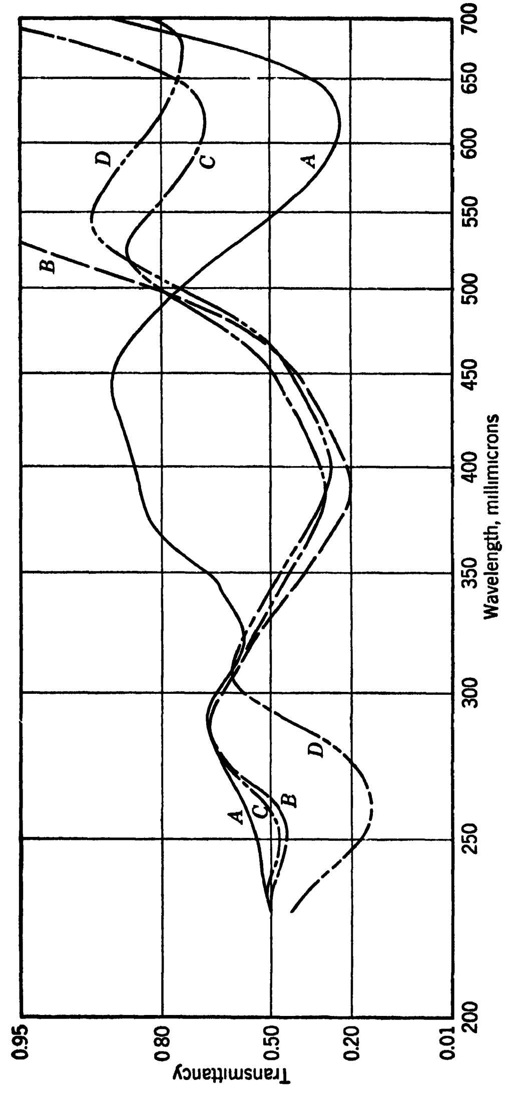

FIG. 7·14. Effect of component interaction on absorption spectrum of mixture of diamine sky blue FF and chrysophenine.

A = diamine sky blue FF (C.I. 518);
B = chrysophenine (C.I. 365);
C = predicted curve of 25 per cent A and 75 per cent B;
D = measured curve of 25 per cent A and 75 per cent B.

All concentrations 0.015 g. l. total dye; all solutions irradiated; solvent = water; a_s = 42 for A at 615 mμ.

and the resulting curve of the mixture will be unexpected. Thus, in Fig. 7·14 curves A and B are the curves of the two components measured individually. The mixture of 25 parts of blue dye and 75 parts of yellow dye would be expected to plot between the two curves of the components at all wavelengths, as shown by curve *C*. For instance, at 250 mμ it would be expected to fall between curves *A* and *B*, but instead it plots at a much lower transmittancy, as is shown by curve *D*. It is evident that without proper appreciation of this lack of additivity the interpretation of curve *D* would lead to erroneous conclusions regarding the identity and quantity of components present.

In the event of lack of additivity, such as that of Fig. 7·14, it is usually much easier to change the solution conditions so that the components are additive than it is to introduce mathematically the correction factors needed to account for deviations from true additivity. In practice this may be accomplished for the dyes of Fig. 7·14 by (1) raising the temperature to boiling, (2) changing the solvent to a mixture of 50 per cent water and 50 per cent pyridine, or (3) adding a surface-active agent to the extent of about 1 per cent. If a suitable surface-active agent is added, the curves obtained will not be like any of those in Fig. 7·14, but the absorption curve of the mixed components in the presence of the surface-active agent will be predictable from the absorption curves of the pure components prepared in the presence of the agent.

7·19 Hydrolysis. Many compounds are not stable in a particular solvent but react with it to give products with an absorption curve of different shape. If this solvation is slow, it may pass unnoticed yet contribute to poor reproducibility of solution measurement. Holmes and Darling (*98*) have called attention to the fact that solvation can exist for such a relatively stable compound as the commercial dye auramine O in such a nonreactive solvent as water. This is illustrated by the data of Fig. 7·15, which shows auramine O dissolved in water and measured immediately, compared to the same solution after being held at an elevated temperature in a slightly acidic solution which induces hydrolysis.

7·20 Light fading. The permanent destruction of a compound under the action of light, with a resultant change in the absorption curve, is known as fading. It is true that any weakening of a color may be called a "fading" of the color, but unless otherwise specified the cause is incident light. Some colorimeters and spectrophotometers which expose the solution to the full light intensity and subsequently filter or monochromate the light are likely to introduce errors of this kind. In an instrument in which the light is monochromated before the sample is irradiated the intensity of the light is usually very low, and fading is ordinarily not a factor.

Storage of the solutions in direct sunlight before measurement may also introduce significant fading. Fading is ordinarily detected by an ageing test and may be distinguished from other effects by a different ageing when stored in the light from that when stored in the dark.

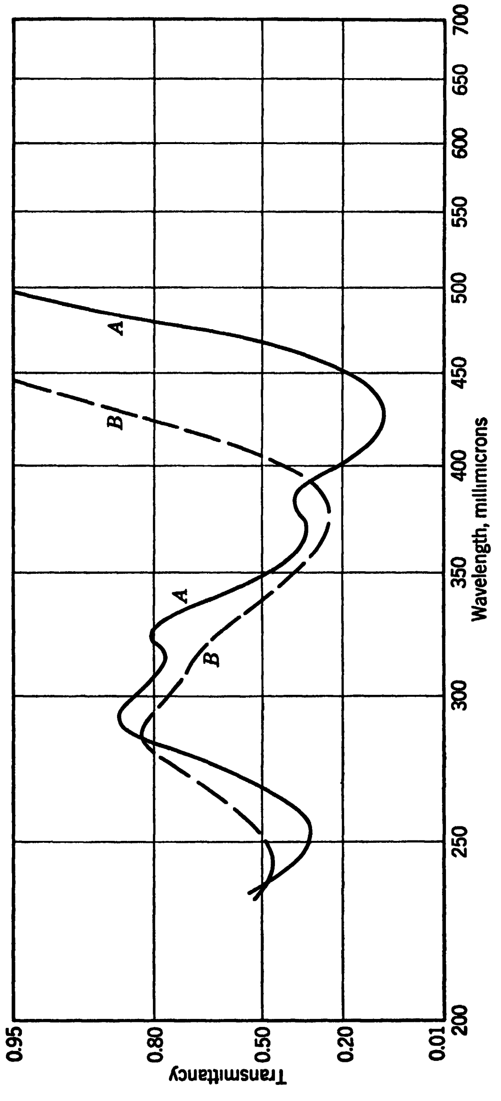

FIG. 7·15. Effect of hydrolysis on absorption spectrum of auramine O (C.I. 655).

A = freshly prepared solution;
B = aged solution.

Solvent = water at low *p*H; a_s = 111 at 425 mμ for *A*.

The fading of solutions has been used to advantage in the quantitative analysis of vitamin A (*126*) and has been studied in an attempt to correlate structure and fastness to light of monoazo dyes (*110*). Figure 7·16 illustrates the fading by light of a monoazo dye in solution.

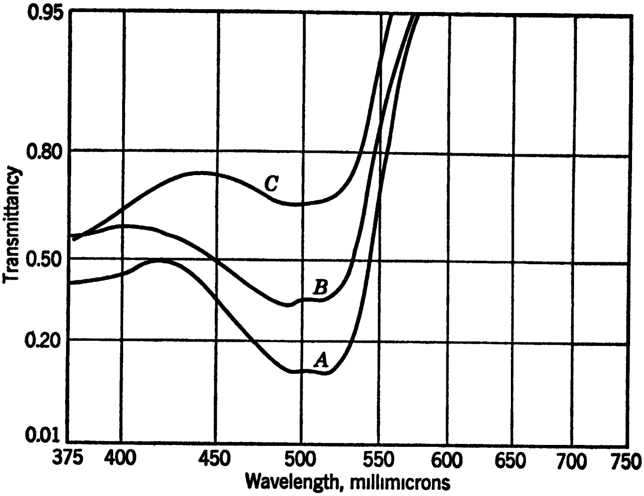

Fig. 7·16. Effect of fading of solutions on absorption spectrum of 2-nitroaniline coupled to R-salt.

A = initial solution;
B = 100 hours' exposure to commercial fading lamp;
C = 200 hours' exposure to commercial fading lamp.

Solvent = water; a_s = 41 at 515 mμ for *A*.

7·21 Fluorescence. Fluorescence is the property of a material which enables it to absorb light and then re-emit it. Ordinarily the re-emitted light is of the same or longer wavelength than the exciting light and usually it is re-emitted randomly in all directions. Franck and Livingston (*71*) have presented a general discussion of the theory.

The commercial dyes which exhibit fluorescence have been reviewed by Millson (*142*), who has pointed out the usefulness of fluorescence in identification and testing. The chemical and physical factors which influence it have been studied (*125*, *155*, *165*, *204*, *205*), together with the physical properties of the emitted light (*211*).

Fluorescence is used in quantitative analysis (*74*). The usual method is to determine an empirical relationship between concentration and fluorescence for a standard material and then to measure the fluorescence of solutions of unknown concentration. Fluorescence is used in qualitative analysis, usually by a visual estimation of color (*99*). Instruments which measure intensity of fluorescence are known as fluorimeters. The intensity of fluorescent light as a function of wavelength may be determined with a spectroradiometer (*217*). The data obtained from both these instruments are outside the scope of this chapter. The phenomenon must be included in this chapter because it is a source of error in absorption spectrophotometry (*76*, *146*) if not recognized.

The reason that fluorescent light introduces an error in spectrophotometric data is that a fundamental assumption of the determination of concentration by absorption of light is that the absorbed light disappears, being dissipated as heat. This assumption is false if the absorbed light can be re-emitted and continue on through the optical system to give a response to the photoreceptor. The best way to minimize the error of fluorescence is to take advantage of the fact that fluorescent light is emitted randomly in all directions, whereas the remainder of the incident light that is not absorbed continues along the optical path of the instrument. Figure 7·17 shows the apparent absorption curves measured with a spectrophotometer having an integrating sphere to collect the light after it passes through the solution. Curve *A* of Fig. 7·17 represents the curve obtained when the solution is placed close to the entrance window of the integrating sphere. A considerable fraction of the fluorescent light finds its way into the sphere and records as an apparent transmittancy. Curve *B* of Fig. 7·17 represents the curve obtained when the solution is placed 10 cm. away from the integrating sphere. Only a negligible fraction of the fluorescent light is incident on

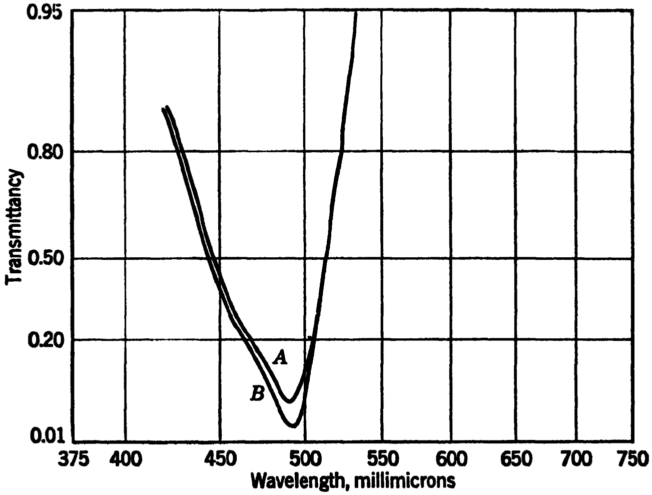

FIG. 7·17. Effect of fluorescence on absorption spectrum of uranine (C.I. 766).

A = solution close to integrating sphere of spectrophotometer;
B = solution 10 cm. from integrating sphere window.

Solvent = water; a_s = 234 at 491 mμ for *B*.

the photocell because of the geometrical arrangement, and curve *B* represents essentially the true transmittancy curve.

7·22 Turbidity. In general, the exponential decrease in intensity of the collimated component of a light beam passing through a solution will involve both an absorbancy coefficient term and a scattering coefficient term. For zinc oxide suspensions the absorbancy coefficient is essentially zero, and the transmittancy curve obtained with a spectrophotometer is a scattering curve rather than an absorption curve. Such curves are useful in interpretations of particle size and distribution, but these data are neither absorption data nor solution data and for both reasons are outside the scope of this chapter.

Unless the scattering coefficient is zero, the apparent absorbancy coefficient will be erroneously high (*137*). It is true that this error will tend to be proportional to concentration, and the experimental data may follow Beer's law; but the difficulties in preparing reproducible suspensions tend to decrease the accuracy of the method.

7·23 Plating. The property of some compounds coming out of solution and depositing themselves on a solid surface is known as "plating out." This property was well known in 1911. Marc (*129*) reported the absorption of various dyes including methylene blue (C.I. 922) and methyl violet B (C.I. 680) on crystals of other materials growing in aqueous solutions saturated with these other materials. This absorption is so rapid that the plating of fluorescein (C.I. 766) on silver chloride can be used as an indicator of an end point in quantitative titrations (*70*).

Use of this property for the analysis of pigments has been recommended by Bancelin and Crimail (*9*), who point out that methyl violet plates out on titanium dioxide but not on barium sulfate, and hence one can determine microscopically by examination of a tinted mixture of these two white pigments the approximate proportion and also whether the mixture was obtained by mechanical blending or simultaneous precipitation. Along the same line of reasoning Bosazza (*19*) has suggested that malachite green (C.I. 657) be used for the analysis of mixtures of clays by virtue of the fact that it is differently adsorbed by various kinds of clay.

Plating effects are not limited to dyes, for it has been noted in biological spectrophotometry that calciferol and animal lipids are adsorbed on crystalline-quartz cell faces (*140*). Various workers in photometry have noticed plating (*46, 67, 165*), and to avoid it the use of alcohol solutions rather than water (*64*) or the use of special glass rather than ordinary soft glass for cell faces has been recommended (*68*).

The plating is apparently the formation of a monomolecular layer of molecules on the receptive surface, after which no further plating occurs. In measuring a series of solutions the first one plates on the cell faces. If the cell is then merely rinsed with solvent, but not scrubbed with soap and brush or rinsed with a powerful cleansing solution, the second solution will have an apparent increase in concentration by the amount that the plated-out material contributes. All subsequent solutions will have the same error, that contributed by the plating of the first. The error may be avoided by plating out both the sample-solution cell and reference-solution cell before beginning to make transmittancy measurements.

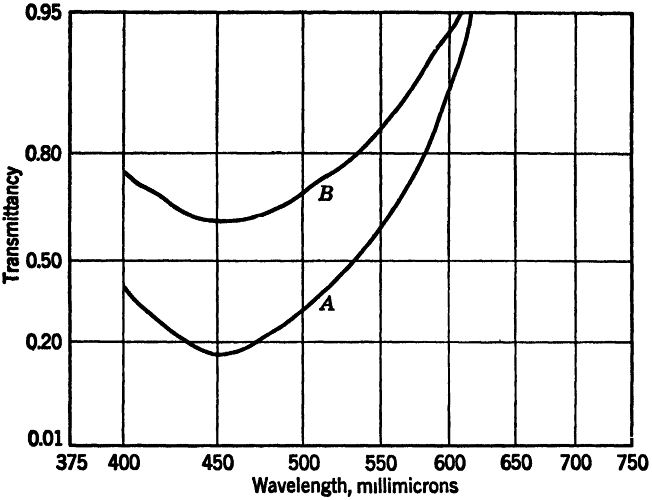

FIG. 7·18. Effect of plating on absorption spectrum of bismarck brown R (C.I. 332).

A = special cell having 28 glass-solution surfaces filled with dye solution;
B = same cell after emptying and rinsing but not scrubbing.

Figure 7·18 illustrates this effect. A thick cell was chosen and thirteen pieces of glass put into it. This cell was filled with a bismarck brown R (C.I. 332) solution and the curve plotted, giving curve *A* of Fig. 7·18. The solution was then poured out, and the cell well rinsed with cold water several times. When the cell was filled with water and measured again, curve *B* was obtained. Although curve *B* shows the characteristic absorption curve of bismarck brown R, the dye was not in solution but was a plated deposit on twenty-eight glass surfaces. Quantitative measurement shows that with an ordinary cell a 2 per cent concentration error would be introduced by failing to take this plating into account. Larger relative errors would result in work with less concentrated solutions.

7·24 Control of factors. Sections 7·8 to 7·23 describe various solution variables which tend to change the shape of the absorption curve. A study of these solution effects comprises one of the applications of spectrophotometric methods. On the other hand, when the purpose of the spectrophotometric measurement is qualitative or quantitative analysis for the components present in the solution, these effects must be recognized and accounted for. Otherwise an uncontrolled solution effect may be misinterpreted as a change in concentration or chemical identity. Standard conditions must be set up for each measurement to insure maximum accuracy.

7·25 Theory of control. An error in observed absorbancy coefficient will depend upon the magnitude of the variation from standard conditions and the sensitiveness of the effect. Thus

$$\Delta k_s = \Delta a \left(\frac{\partial k_s}{\partial a}\right) d, g + \Delta d \left(\frac{\partial k_s}{\partial d}\right) a, g + \Delta g \left(\frac{\partial k_s}{\partial g}\right) a, d, \cdots \qquad (7\cdot 7)$$

where Δk_s = the error in the observed value of the absorbancy coefficient at any one wavelength.

Δa = the difference in solution condition a from standard.

$\partial k_s/\partial a$ = the sensitiveness of the absorbancy coefficient to a change in solution condition a.

a, d, g = various solution conditions.

To keep Δk_s small it is necessary to have each term in equation (7·7) small. The measurement conditions should be chosen so that this is true.

Figure 7·5 shows the absorption curves of bromothymol blue at various *p*H values. If it is desired to determine the concentration of bromothymol blue in a solution by measurement of the absorbancy coefficient at 610 mμ, the *p*H must be controlled. For this example, in equation (7·7) Δk_s is the error in the measured absorbancy coefficient at 610 mμ, which should be kept small. If solution condition a in equation (7·7) is the *p*H effect, one way to keep the first term of the equation small is to keep the term Δa small. This means that the *p*H of the solution of unknown concentration should not deviate much from the *p*H of the standard condition. If the standard condition requires measurement of the absorbancy coefficient at *p*H = 3.00, the sample should be held closely to 3.00. This means it is advisable to add a buffer.

Another way to keep the first term of equation (7·7) small is to keep $\partial k_s/\partial a$ small. This means working at a *p*H where the change in absorbancy coefficient with change of *p*H is small. Figure 7·5 shows part of the data of bromothymol blue taken in an initial study to choose standard conditions of measurement for quantitative analysis. Figure 7·19 shows the absorbancy coefficient at 610 mμ as a function of pH. Also in Fig. 7·19 is plotted the first derivative of this curve which is $\partial k_s/\partial a$. The first derivative is small over the range of 1 to 5 pH and over the range 10 to 12 pH. For accurate evaluation of k_s it is better to work in one or the other of these pH ranges of low sensitiveness.

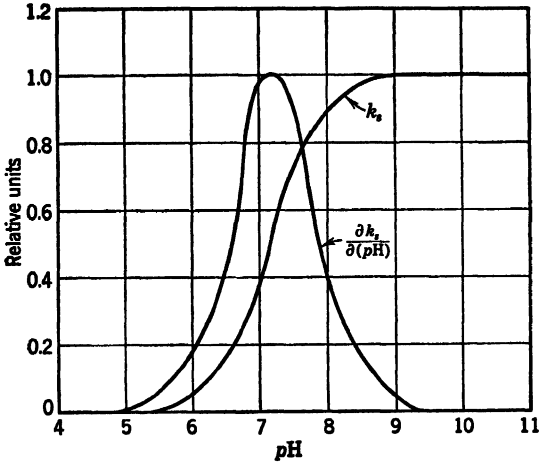

FIG. 7·19. Effect of hydrogen-ion activity (pH) on absorbancy coefficient (k_s) of bromothymol blue at 610 mμ.

If the standard conditions call for buffering to a pH of 3.00, the contribution of pH to an error in k_s should be negligible because the combination of small sensitiveness (small $\partial k_s/\partial a$ because a pH of 3 is chosen) and small deviation from standard conditions (small Δa because the solution is buffered) should make the pH term of equation (7·7) small.

Sometimes it is not desirable to work in regions of low sensitiveness to a particular effect, perhaps because the phenomenon being studied does not occur under these conditions. In such a case the relation of the absorbancy coefficient to the effect must be determined by experiment. It is also necessary to measure the deviation of the solution of unknown concentration from the standard condition as regards the effect under consideration. Then the correction Δk_s may be calculated.

Whether the purpose is to choose ideal measurement conditions so that effects may be minimized by control, or to prepare to correct for deviations in the effect from standard conditions, it is necessary to determine the influence of various solution effects to obtain maximum accuracy in analytical work.

Another method of keeping the terms of equation (7·7) small, which may be used if necessary, is to take several measurements and extrapolate to $\Delta a = 0$. As an example, the work of Duke (*66*) may be cited. The solution effect involved was fading. Thus a in equation (7·7) was time of fading. The analysis was handled successfully by measuring the absorbancy coefficient at various times, plotting a curve, and extrapolating to determine the absorbancy at zero time. As another example, but one in which the solution effect was oxidation, the work of Mindlin and Butler (*145*) may be cited. In this system the dye remaining after reduction by the desired component was unstable because an impurity reduced the dye slowly. This oxidation effect was eliminated as a source of error by plotting absorbancy versus time and extrapolating to zero time.

7·26 Test for stability. Some solution variables more frequently affect the absorption-curve shape than do others. The particular factors which are most important in any group of materials should be determined, and when the ideal measurement conditions according to the principles outlined in Section 7·25 have been chosen, it is probable that satisfactory conditions have been found. Two tests are desirable to check this, reproducibility and ageing. If the reproducibility of solution preparation and measurement is good, and if the ageing is insignificant, satisfactory conditions have been found.

If the reproducibility and ageing are poor, a further study should be made to determine the cause. At this time the less frequently important factors should be methodically investigated. The reproducibility tests should be carried out preferably by replicate measurements on different days, as replicate measurements on the same day are likely to give erroneously good indications of precision. Some important variable (unrecognized) is more likely to be encountered. In analytical tests, 24-hour ageing is ordinarily sufficient, since measurements are usually made on the solutions before they are that old. On the other hand, in studying some slow reactions, an ageing test over the period of a month may be necessary to exceed the time during which the solutions are under study.

7·27 Optimum solution transmittancy. The problem of primary interest in quantitative analysis is to determine concentration, c, with a minimum percentage error. The percentage error will vary, depending upon the transmittancy of the solution. Hence a problem in choosing standard conditions is to select the concentration and cell length so that an optimum transmittancy will result at the wavelength of calculation. One of several equally important factors which determine an optimum transmittancy is instrument performance.

For purposes of illustration, the optimum transmittancy of a solution for measurement with a spectrophotometer employing a Martens photometer and electrical balancing will be calculated. If the recording pen is forcibly displaced from the true balance point, it will record an erroneous transmittancy which will give, upon calculation of concentration from transmittancy, a percentage error $\Delta c/c$. Such a forcible displacement will also give rise to an electrical signal Δs, which will tend to restore the pen to its true balance point. For maximum accuracy it is necessary to have the ratio of the strength of the restoring force to the error in percentage concentration a maximum, that is, to have $c\Delta s/\Delta c$ a maximum. The plot of interest is therefore a plot of $c\Delta s/\Delta c$ versus T_s so that an optimum T_s may be chosen.

Beer's law, equation (7·1), relates c to T_s. In a Martens photometer the relation between transmittancy and angular rotation of the Rochon prism is given by

$$T_s = \tan^2 A_0 \tag{7·8}$$

where A_0 = angular position of the Rochon prism. The relation between angular position of the Rochon prism and the excess light in the reference beam is given by the laws of polarizing prisms as follows:

$$\Delta L = \sin^2 A - T_s \cos^2 A \tag{7·9}$$

where ΔL = the excess light in one beam which may be called the optical signal tending to restore balance. When $\Delta L = 0$, $A = A_0$. Assuming an electronic amplifier in which the electrical signal is directly proportional to the optical signal,

$$\Delta L = \Delta s \tag{7·10}$$

From equations (7·1), (7·8), (7·9), and (7·10) one may arrive at a single equation as follows:

$$\frac{c\Delta s}{\Delta c} = \frac{T_s\, 2.303 \log T_s}{1 + T_s} \tag{7·11}$$

It may be shown that maximum $c\Delta s/\Delta c$ occurs at $T_s = 0.28$, but it is more useful to plot $c\Delta s/\Delta c$ versus T_s, as is done in Fig. 7·20. This shows that large ratios are obtained over the range 10 to 60 per cent transmittancy.

Other types of spectrophotometers will have other optimum transmittancy values and curves. For instance, a type which operates on the principle of setting a solvent-filled reference cell to read 100 per cent, followed by electrical balancing with a linear potentiometer to read the transmittancy of the solution, will have an unbalance signal which is directly proportional to the absolute transmittancy unbalance. For this type the optimum transmittancy will be found to be 36.8 per cent (*83a*).

A further complication is that some popular instruments have a modification so that the range 0 to 10 per cent or 0 to 20 per cent transmittancy is made to read full scale. These magnifications make it easier to read the transmittancy values, and minimize some mechanical defects such as paper positioning and pen wobble in recording instruments, or mechanical play in potentiometer contact points and slide-wire irregularities in indicating instruments, but do not change the ratio of $c\Delta s/\Delta c$. It is unfortunate that these magnifying modifications in general are not designed to magnify the transmittancy range of greatest accuracy in analytical work. Figure 7·20 has been drawn subject to the implicit assumption that an equal electrical unbalance of the instrument can be detected with equal precision at all transmittancies. The magnifying modifications mentioned above tend to make this assumption inapplicable. A complete discussion of the optimum range of each instrument is outside the scope of this chapter, but the recognition of this optimum range is of importance in considering three questions relating to analytical applications: (1) What transmittancy should a solution have for analytical purposes? (2) What is the useful range of concentrations of an analytical method? (3) What is the ultimate accuracy of spectrophotometric analyses?

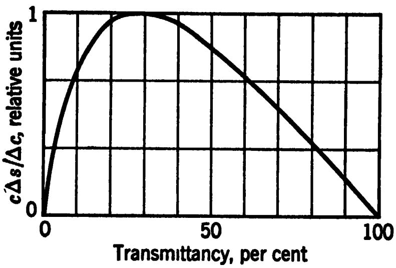

Fig. 7·20. Optimum solution transmittancy for a spectrophotometer with electrically balanced Martens photometer.

In considering the question of the transmittancy of a solution for analytical purposes, a curve such as Fig. 7·20 should be calculated and the transmittancy limited to a range where the ordinate is large. In addition, a low transmittancy is to be avoided because of stray light in the instrument, poor solubility, and less probability that Beer's law will be obeyed. A high transmittancy is to be avoided because of accidental dirt such as fingerprints on the solution cells, inaccuracy in the calculated transmittance of the water-filled reference cell or blank, greater influence of turbidity of solvent, and need for more volumetric dilutions in preparing a sample which must be a given weight to prevent sampling errors. As a general statement, the transmittancy range 10 to 50 per cent is usually satisfactory for accurate quantitative work.

The useful range of concentrations of an analytical method is often stated in the literature. For example, a range may be given as 3 to 30 mg./l., based on the fact that the final solution, measured in a 1-cm. cell and measured near the wavelength of the absorption maximum, falls in the range of 15 to 60 per cent transmittancy which is a region of high $c\Delta s/\Delta c$ for a particular instrument. If, however, it were desired to measure weaker solutions, this could be done by measuring them in a 10-cm. cell. In this case the optimum range would be from 0.3 to 3 mg./l. Further, if it were desired to measure more concentrated solutions without diluting them, this could be done by measuring at a wavelength where the absorbancy index was one-tenth that of the maximum. Alternatively a 1-mm. cell could be used. In either case the optimum concentration range would be 30 to 300 mg./l. Of course the validity of Beer's law should be checked over any concentration range that is recommended for analysis. Thus the extension of an analytical method over the concentration range of 1 to 1000 or greater may be accomplished without any loss in accuracy, provided only that the cell length and wavelength of measurement be chosen so that the measured transmittancy falls in an optimum range as determined by a plot such as that of Fig. 7·20.

In considering the ultimate precision of photometric methods, so much depends on the particular instrument used that no general answer can be given. Let it first be assumed that the instrument is capable of a precision of 0.2 per cent transmittancy, that the measurement of the solution is made relative to the solvent (this assumption is inherent in the definition of the word *transmittancy*), and that the solution has a transmittancy of 30 per cent. Then it can be shown on the basis of Beer's law that approximately 0.5 per cent concentration change is the ultimate precision. Many routine analyses are carried out in this manner, and many spectrophotometers are precise to about this extent, so that 0.5 per cent concentration is a general statement for routine analyses. Changing the cell length, changing the wavelength of analysis, or changing the solution concentration will not reduce this figure but may increase it. However, if it is permissible to widen the slit width of the instrument, or otherwise increase the amount of light, the precision can be greatly improved by determining a ratio of transmittances of a solution to a standard. Suppose that orange II, the curve of which is shown in Fig. 7·1, has a transmittancy of 30 per cent at a concentration of 10 mg./l. at a given wavelength in a given cell. In the conventional measurement with one type of spectrophotometer a water-filled cell is placed in the light beam and the slit width adjusted until the null point is indicated at 100 per cent transmittance. Then the solution is placed in the beam and the null point is indicated at 30 per cent. But these spectrophotometers can also measure ratio of transmittance, R_T, for instance transmittance of 20 mg./l. solution divided by transmittance of 10 mg./l. solution. In this case

$$R_T = \frac{10^{-a_s bc_1}}{10^{-a_s bc_2}} = 10^{-a_s b(c_1 - c_2)}$$

where c_1 is the concentration in the sample measurement, and c_2 is the concentration in the reference measurement. The maximum precision for this type of measurement is a function of both c_2 and R_T. In general, c_2 should be large and R_T should be greater than 0.368; indeed, for some values of c_2, it should be as large as 1.00. In the Beckman spectrophotometer, for instance, the operating procedure is to place the c_2 solution in the light beam, adjust the slit width until the transmittance reads 100 per cent, and then read the c_1 solution. The limit is reached when c_2 is so great that the mechanical limitations of the slit do not permit further widening. At wavelengths of good sensitivity this occurs when c_2 is quite large, and the result is that the relative error in c_1 can be reduced well below 0.1 per cent. A spectrophotometer could be designed to have a still wider slit. The question then becomes, what is the widest slit that an instrument may have and still be called a spectrophotometer? As a general statement, in answer to the question of ultimate precision, it may be said that the precision is often no greater than 0.5 per cent but may easily be reduced to 0.1 per cent if desired.

It may be mentioned that in this procedure it is quite common to use a strongly absorbing glass filter rather than a solution c_2, but the theory is unchanged. This method of measuring one solution against another has other advantages than improved precision. These are discussed in Section 7·40.

D·QUALITATIVE ANALYSIS

One of the major advantages of spectrophotometry over filter photometry is its application to qualitative analysis. Filter photometric data, consisting of single transmittancy values at single wavelengths, are not diagnostic. Spectrophotometric data, consisting of the transmittancy as a function of wavelength over a range of wavelengths, are characteristic curves which are "signatures" of the compounds.

The identification of compounds by the shape of their absorption curves is empirical. It is accomplished by finding the curve of a known compound that agrees with the curve of the unknown. Perhaps some day the correlation of structure and absorption-curve shape will be established, and it will be possible to measure an unknown and determine its structure without reference to other curves. This has already been done when the identification is limited to a particular and highly restricted group of compounds (*35*), but for general identification it appears that the approach will always be empirical.

In spite of its empirical nature, spectrophotometric identifications are widely accepted in many fields, provided the work is carefully done (*73*, *105*, *159*, *169*).

7·28 Identification by curve shape. In connection with the use of transmittancy-wavelength curves for purposes of qualitative analysis, one's concern is with the contour or shape of the curve. Two attributes are of primary concern: (1) what maxima and minima occur and, sometimes, their relative heights; and (2) the position of such maxima and minima with respect to wavelength.

As pointed out in Chapter 2, various systems of plotting spectrophotometric data are being, or have been, used. For the applications discussed here, the writer believes in the system evolved to handle the very extensive collection of curves compiled in his laboratory.

7·29 One component. Because spectrophotometric identifications are empirical, the data must be carefully evaluated to determine the certainty with which the identification is established. It must be remembered that there are some structural differences which introduce essentially no change in the spectral absorption curve over a limited range of wavelengths. An identification based on agreement with the curve of a known compound is at best only the identification of a chromophore group. Even the identification of the chromophore group may be in error if the data are available over only a small range of wavelengths. For instance, the curve of potassium chromate can be made to agree closely with the curve of an entirely different compound developed in a test for silica, provided the observation is limited to the range 440 to 500 mμ (*193*). On the other hand, if two compounds have identical curves over the entire range of ultraviolet, visual, and infrared absorption, the identification is much more certain.

A second way to improve the certainty of the identification is to determine the curves of the unknown and known of suspected identity in several reactive solvents, or after the compounds have been subjected to chemical reactions which change their absorption curve. Figure 7·3 shows the curves of indigo MLB/2B in four solvents. If an unknown dissolved in pyridine shows a curve shape similar to curve *D* of Fig. 7·3, it would be suspected that the unknown is chemically identical to indigo MLB/2B, at least with respect to its principal chromophore group. On the other hand, if the unknown agreed with the known as regards curve shape in all four solvents of Fig. 7·3, the evidence of chemical identity would be very strong.

A third way to improve the certainty of identification is to determine whether the unknown and known have similar solution effects. Thus, if both a known and an unknown have a *p*H effect like that of Fig. 7·5, or a temperature effect like that of Fig. 7·7, or an irradiation effect like that of Fig. 7·11, the evidence of chemical identity is strengthened.

A fourth check involves a solubility method. If both known and unknown have identical curves, and identical absorbancy coefficients at maximum solubility, as a further test a mixture of the two in equal proportions may be made up in solution. If this mixture also has an identical curve, and identical absorbancy coefficients at maximum solubility, it is likely that the known and unknown are identical. If the compounds are different, they are likely to dissolve more or less independently of each other, and the absolute values of the absorbancy coefficients of the mixture should be higher.

A fifth criterion of certainty involves the complexity of the curves. If the curve of the unknown has many points of inflection, and this curve is found to agree with a known, the identification is more certain than if agreement is found between two curves that are relatively straight.

In all comparisons of curve shapes of unknown and known, it is necessary that the solution effects of Sections 7·8 to 7·23 be controlled. Also, it is practically impossible to make rapid comparisons of curve shape for purposes of identification unless all the standard data are plotted on a log A_s ordinate. If this ordinate is not used, some special experimental conditions must be specified, such as having 10 per cent minimum transmittancy for all curves, or transformations must be computed to reduce the curves to equivalent form. It is also much more convenient if the curves are plotted on transparent paper, as this makes it possible to superimpose the curves of unknown and standard. With two superimposed curves the significant similarities or differences may be quickly observed.

It is also important not to fail in an identification of two materials which are essentially chemically identical because of trivial differences arising from small amounts of impurities. Impurities are more likely to cause a greater change in curve shape at regions of high transmittancy. Hence, for reports of "essentially chemically identical," small variations in regions of high transmittancy may often be ignored.

The ability to identify unknowns presupposes a file of standard curves of known compounds. A rapid identification from a large file of standard curves presupposes a satisfactory curve-shape index. The curve-shape index should enable one to go directly to the few curves in the file of standards which might be a match for the unknown. Then a direct comparison of individual curve shapes should be made as final evidence of identity.

An excellent curve-shape index has been reported by Shurcliff (*176*). In this system the most important characteristic of identity is considered to be the wavelength of the absorption maxima. If an absorption curve has three or more absorption maxima in the spectral range covered by the index, the first number in Shurcliff's system is a 3, which indicates this fact. The second number in this system is the wavelength of the absorption maximum which has the greatest absorbancy index, the third number is the wavelength of the absorption maximum which has the second largest absorbancy index, and the fourth number is the wavelength of the absorption maximum with the third largest absorbancy index.

The second most important characteristic of identity in Shurcliff's system is the ratio of absorbancy index at different wavelengths. In the case of compounds having an absorption curve with only two peaks, the system calls for an index of a first number 2, indicating a two-peak curve, a second number which is the wavelength of the absorption maximum having the larger absorbancy index, a third number which is the wavelength of the absorption maximum having the smaller absorbancy index, and a fourth number which is the ratio of the smaller absorbancy index to the larger.

In the case of curves with a single maximum, the index has as its first number 1, which indicates the one-peak curve, and as its second number the wavelength of this peak. The third number is the wavelength longer than that of the absorption maximum at which the ratio of the absorbancy index to the absorbancy index of the maximum is 0.5. The fourth number is the wavelength shorter than that of the absorption maximum at which the ratio of absorbancy index to the absorbancy index of the maximum is 0.5.

As a further characterization of single-peak curves, it is sometimes necessary to use the wavelength where the ratio of absorbancy index to that at the absorption maximum is 0.1.

A curve-shape index must be adapted to the practice within a particular laboratory. Thus, for bromothymol blue as illustrated in Fig. 7·5, the absorption curve may be considered to be a three-peak curve if the entire spectral region shown in the figure is considered, or it may be considered a one-peak curve if only the visual portion of the spectral range is considered. If the standard practice in a laboratory is to obtain the entire curve shown before attempting to interpret the data, the three-peak symbol is better. If the standard practice is to obtain the visual range on one instrument, perhaps an automatically recording one, and supplement the curve with the ultraviolet range from another instrument, perhaps a manually recorded one, it may be better to have two separate indexes, one limited to the visual range and classing the curve as one-peak, the other limited to the ultraviolet range and classing the curve as two-peak. Then, when the data from either instrument are available, a tentative identification may be made, the identification becoming more certain when the data from the other instrument substantiate it.

It is also obvious from a consideration of Fig. 7·5 that the solution conditions must be specified. If it is standard laboratory practice to measure unknowns in two solutions at different *p*H values, the curve-shape index should be first divided into solvents, and water of *p*H 3 should be considered as one solvent, water of *p*H 9 as another. Use of different solvents will enlarge the index but will also give more certain identifications.

The curve-shape index will then be classified first by spectral range, second by solvent, and third by curve-shape numerical symbol. The practice within a laboratory will determine the spectral range and solvents of interest. Other details will also lead to slight variations. If the visual range is considered to extend from 375 to 750 mμ, a different symbol may apply from that chosen if the range is considered as 400 to 700 mμ. The different spectral resolutions of different instruments will give different positions to the wavelengths of the symbol. One laboratory may decide that absorption maxima having an absorbancy index less than 0.1 the value of the absorbancy index at the major absorption maximum are likely to be caused by accidental impurities and will disregard them. This may change the classification from a two-peak curve to a one-peak curve. Because of these variations, it seems that each laboratory should set up its own index. In any event, correspondence of curve-shape index is not an identification; it is only a means of choosing from the many available curves a few which are most likely to correspond to that of the unknown.

7·30 Several components. A file of standard curves and the corresponding index are necessarily limited to single compounds except for a few cases such as azeotropes, in which two or three compounds are frequently found together in a definite proportion. One method of analyzing mixtures qualitatively is to separate the components physically, as by the use of selective adsorptive procedures (*189*). Another method which may be possible is to destroy all but one compound chemically so that it alone absorbs in the spectral range of interest. For instance, it may be possible to adjust the *p*H so that one compound does not absorb and another one does. Both these methods are directed toward obtaining the curve of only one component so that the curve shape may be used to identify it.

If the components cannot be separated, it is sometimes possible to identify one of the components present by a particular absorption maximum, calculate the absorption curve which would result if this component were absent, and then identify further components. To identify the first component it is advisable to consider the absorption maximum at the longest wavelength first. At short wavelengths all the components are likely to have absorbancy indexes of some magnitude, but at long wavelengths some of the components are likely to have negligible absorbancy indexes. This means that the absorption maximum at the longest wavelength is most likely to be caused by only one component and is the one most likely to be identifiable with a file of single-component standard curves. The absorption maximum at the longest wavelength should be compared with all curves having maxima in this region and considered for agreement in the long-wavelength regions only. Differences in the short-wavelength regions may later be found to be caused by other components. The curve-shape index will not restrict the comparisons to as small a number of standard curves as it will for single components but is still very useful. If the long-wavelength portion of the curves can be made to agree, the identified component is assumed to be present at a concentration equal to the minimum $(k_s)_m/(a_s)_a$, where $(k_s)_m$ is the absorbancy coefficient of the unknown and $(a_s)_a$ is the absorbancy index of the identified component. This amount of identified component is then subtracted graphically from the curve of the unknown by the method described in Section 7·4, and the resulting curve will represent the unknown mixture as if it had been determined after the identified component had been physically removed. The analysis is repeated, an attempt being made to identify the absorption maximum at the longest wavelength of the remaining curve.

The above procedure assumes the principle of additive absorbancies, which is not always fulfilled. Figure 7·14 shows an exception to additive absorbancies. Hence, in any analysis of mixtures it is always well to mix together the suspected components in the suspected ratio and compare the curve of this known mixture with the unknown. Again, in this comparison, the certainty of the identification is increased by greater spectral range, agreement in several reactive solvents, similarity in behavior when subjected to various solution effects, and agreement of complex curves.

7·31 Identification by chemical reactions. Some of the most convincing identifications have been made by carrying out similar chemical reactions with the unknown and with a known compound suspected to be of identical composition, and showing that the absorption curves of the initial samples are alike and the curves of the final samples are alike. The exact procedure to be followed is different in each case.

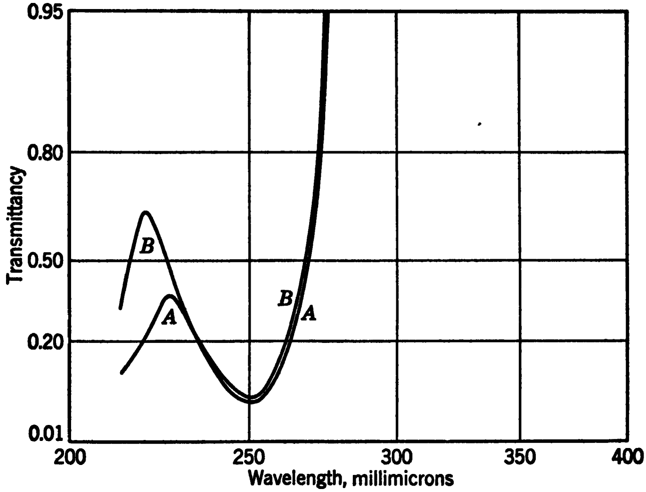

FIG. 7·21. Curves for identification of basic fragment of thiamine.

A = hydrochloride of basic cleavage product;
B = 4-methylthiazole hydrochloride.

Solvent = water; a_s = 23.5 at 252 mμ for *A*; see Ruehle (*169*).

The determination of the structure of thiamine is an outstanding example of the usefulness of spectrophotometry in qualitative analysis. This work was largely done by Williams and Ruehle and their colleagues (*209*) and has been summarized by Morton (*154*). When natural vitamin B_1 was treated with sodium sulfite, it split into two fragments, an acidic fragment and a basic fragment, each with a characteristic absorption curve. The basic fragment, on reaction with hydrochloric acid, was found to have an absorption-curve shape similar to that of 4-methylthiazole hydrochloride, as shown in Fig. 7·21. This was an identification based on curve shape. In addition, since the curves were run quantitatively on fairly pure compounds, the similarity of the absorbancy coefficient values was further evidence of the identity. As still further evidence, the identification was substantiated by a chemical reaction. The basic fragment was reacted with methyl iodide, which introduced a considerable shift in the absorption maximum. The known 4-methylthiazole ethiodide again coincided closely with the basic fragment methyl iodide in both absorption-curve shape and quantitative value of the absorbancy indexes, as shown in Fig. 7·22. This similarity of spectra of two compounds both before and after chemical treatment is very strong evidence that the chromophore group in each is the same.

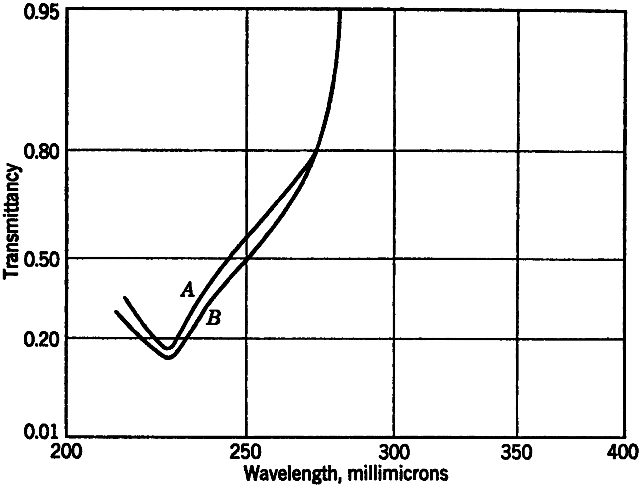

FIG. 7·22. Curves for identification of basic fragment of thiamine.

A = methiodide of basic cleavage product;
B = 4-methylthiazole ethiodide.

Solvent = water; a_s = 43.8 for A at 227 mμ; see Ruehle (*169*).

The acidic fragment resulting from splitting natural vitamin B_1 with sodium sulfite was an aminosulfonic acid which lost ammonia by hydrolysis to yield an oxysulfonic acid. This reaction changed the absorption curve from one having an absorption maximum at 240 mμ to one having maxima at 229 and 275 mμ. This change, shown in Fig. 7·23, is characteristic of the changes produced in aminopyrimidines by hydrolysis, as illustrated in Fig. 7·24 by the known compound, 2,4-dimethyl-6-aminopyrimidine, which hydrolyzes to 2,4-dimethyl-6-oxypyrimidine. Although the curves of Figs. 7·23 and 7·24 do not correspond exactly, the similarity of the curve-shape change, when treated in the same way, shows that the compounds are closely related although not identical. With the assistance of additional work on a cleavage product of thiamine in liquid ammonia, the acidic fragment was finally identified as 2,5-dimethyl-4-aminopyrimidine.

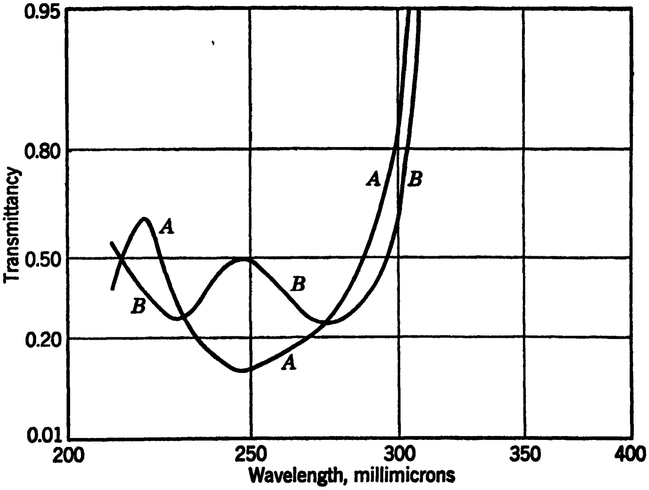

FIG. 7·23. Curves characterizing acidic fragment of thiamine.

A = acidic fragment;
B = hydrolyzed acidic fragment.

Solvent = water; a_s = 49.2 for A at 246 mμ; see Williams *et al.* (*208*).

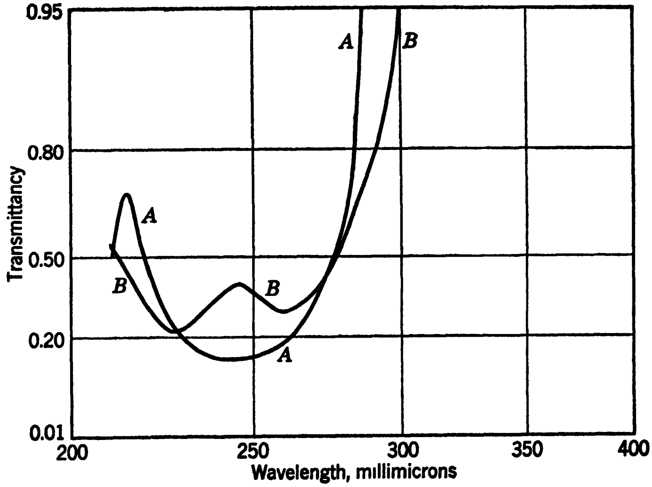

FIG. 7·24. Curves characterizing 2,4-dimethyl-6-aminopyrimidine. (Note similarity to Fig. 7·23.)

A = 2,4-dimethyl-6-aminopyrimidine;
B = product of hydrolysis of A.

Solvent = water; a_s = 44.2 for A at 241 mμ; see Williams *et al.* (*208*).

Thiamine was then synthesized by coupling the thiazole and pyrimidine portions through the 5-methyoxy linkage of the pyrimidine and the nitrogen of the thiazole. The absorption curves of the natural and synthetic products are closely alike, as shown by Fig. 7·25.

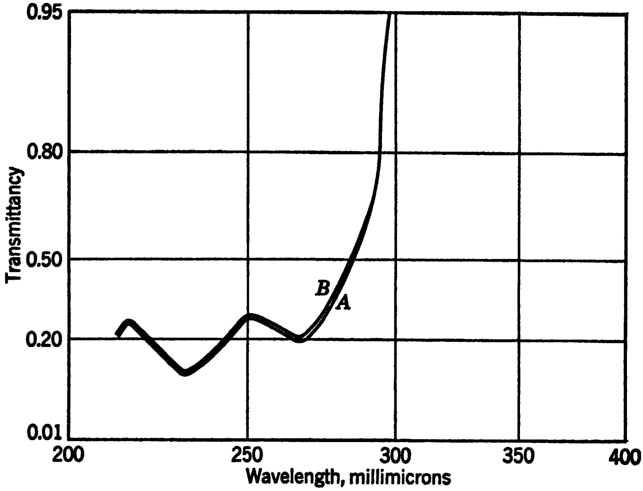

FIG. 7·25. Curves for natural and synthetic thiamine.

A = natural vitamin B_1 hydrochloride;
B = thiamine hydrochloride.

Solvent = water; a_s = 31.2 for A at 232 mμ; see Cline *et al.* (*48*).

E·QUANTITATIVE ANALYSIS

Quantitative determinations involve merely the solving of simple equations if the absorbancies of the solutions under consideration are additive. Absorbancies are additive for one component if there is a linear relationship between concentration of component and absorbancy of solution. Absorbancies are additive for mixtures of two or more components if the sum of the absorbancies of the individual components equals the absorbancy of the solution of the mixture. If absorbancies are not additive, quantitative determinations are empirical and depend upon prior determination of transmittancy-concentration relationships.

The determination of concentration from transmittancy values involves solving certain equations or having reference to empirical graphs in the event that the relation between absorbancy coefficients and concentrations is not linear. Determination of concentration by these methods is called the algebraic method. In the event that absorption-curve data are available on transparent paper with log A_s ordinate and a transparent logarithmic reference scale is available, all of which were described in Section 7·3, determinations may be carried out nomographically. Fundamentally the mathematics is the same, both methods requiring solution of the same equations, but the actual technique differs so much that the latter method, which may be called the log A_s method, is treated in separate sections.

7·32 One component. Great numbers of determinations in routine industrial and clinical laboratories involve only one desired absorbing constituent. The principles of making such determinations, together with specific examples, are described in succeeding sections.

The one-component method, at least in the simpler situations, is the type most likely to be feasible with a filter photometer.

7·33 Measurement of self-colors and developed colors. Sometimes two methods of analysis are available, one the direct measurement of a compound and the other the measurement of the absorption of a compound developed from the first by a chemical reaction. For instance, the analysis of Tobias acid may be carried out by the direct measurement of its ultraviolet absorption, as illustrated in Fig. 7·26, or the Tobias acid may be diazotized and coupled to beta-naphthol to give a red azo dye which may be measured in the visual range, as illustrated by the data of Fig. 7·27. The better method is the one which gives the better reproducibility and greater convenience. In the measurement

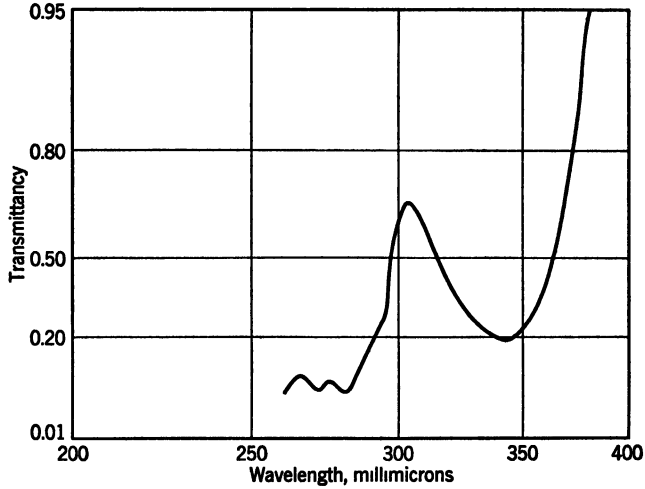

FIG. 7·26. Curve for direct measurement of tobias acid in the ultraviolet. Solvent = water; a_s = 14.4 at 342 mμ.

of vitamin A in butter, instead of the direct ultraviolet measurement, it is better to develop a visual color with a chemical reaction to avoid the effect of yellow coloring materials added to the butter in the process of manufacture (*216*). If everything else is equal, the self-color is probably the better because less work is required in the preparation of the sample.

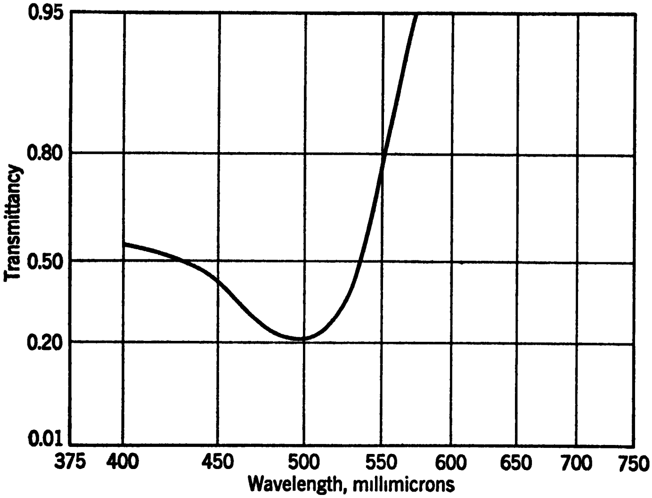

FIG. 7·27. Curve for developed color measurement of tobias acid. Diazotized and coupled to beta-naphthol; solvent = water.

7·34 Choice of wavelength. If the solution of a compound follows Beer's law, its absorbancies are additive at all wavelengths, and theoretically any wavelength may be chosen to evaluate concentration. All should give identical results. There are, however, at least six factors which influence choice of wavelength: (1) wavelength errors, (2) transmittancy errors, (3) solution effects, (4) magnitude of absorbancy coefficient, (5) resolution, and (6) effect of impurities.

In the prism or grating type spectrophotometers a wavelength setting is made at the desired point for analysis and the transmittancy determined. It may be difficult to set such an instrument to get exactly the same wavelength setting. If the transmittancy of a solution is changing rapidly with wavelength over a particular wavelength interval, slight errors in wavelength positioning will introduce an error in the transmittancy determination. If, on the other hand, the transmittancy is not changing over the interval in question, slight errors in wavelength will not matter, as the same transmittancy value will be obtained. This means that the plot of transmittancy versus wavelength should be flat, a condition that holds at the absorption maximum.

The optimum transmittancy of a solution to minimize percentage concentration errors has been discussed in Section 7·27. In addition

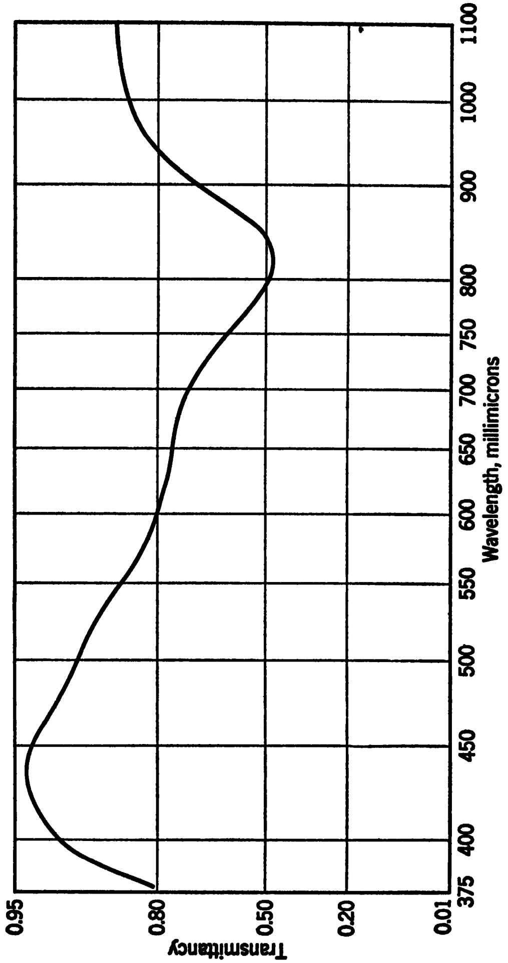

Fig. 7·28. Curve for color development test for silica by the molybdenum blue reaction.
Solvent = water; a_s = 316 at 820 mμ.

to this factor, because of the spectral sensitivity of an instrument, the errors in transmittancy determinations will vary with wavelength. In general, transmittancy can be determined with greater accuracy in spectral regions where the photoreceptor is most sensitive. Thus, in visual spectrophotometry it may be well to choose a wavelength near the maximum sensitivity of the eye at 550 mμ. Figure 7·28 shows the absorption curve of a blue color developed with silica and used for quantitative analysis. Although its absorption maximum falls at 820 mμ, it has been determined visually (*41*), and in various literature reports it is determined by measurement at various wavelengths, depending upon where the sensitivity of the particular instrument falls off in the red end of the spectrum (*22*, *158*, *190*).

A third factor that influences choice of wavelength is solution effects, particularly Beer's law failure. With formyl violet S4B, as Fig. 7·6 indicates, there is only one wavelength near the absorption maximum where absorbancies are additive. It is convenient to choose this wavelength, as additive absorbancies simplify concentration calculations. Similarly, isosbestic points may be chosen to minimize certain effects (*191*). Bromothymol blue measured at 501 mμ would be free from *p*H variations, as Fig. 7·5 shows.

A fourth factor is magnitude of absorbancy index. This is of importance when it is desired to detect very small amounts of a material. Theoretically the use of a long solution cell would make the absorbancy large enough to detect any desired small amount of absorbing material. In practice the length of the solution cell is often limited by the mechanical construction of the spectrophotometer. In general, this factor favors the choice of the absorption maximum.

A fifth factor is resolution. Absorbancies are more likely to be additive with a narrow slit width, and this means working in a region of the spectrum where the available instrument has the greatest resolution. This is not of consequence if the absorbancy indexes are constant over the slit interval.

A sixth factor which influences choice of wavelength is working at a region which minimizes the effect of impurities or of foreign color which is found in the "blank." It is desirable to have a large ratio of absorbancy index of component to absorbancy coefficient of the impurities. Such a choice of wavelength is discussed in Section 7·50. Silverthorn and Curtis recommend working at a very steep portion of the absorption curve of potassium permanganate in order to minimize the effect of the potassium dichromate which is also present (*178*). This shows how one criterion of choice of wavelength, a flat curve, must be sacrificed to satisfy another criterion, minimum effect of impurities.

To review the above six factors, it will be noted that number 4 favors the absorption maximum. Factors 1 and 5 favor an absorption minimum or maximum. Instead of automatically choosing the absorption maximum as the correct wavelength for concentration determination, all the above factors should be carefully considered before making a choice.

In special cases other factors may influence the choice of wavelength. For instance, if the solution concentration and cell length are fixed by the conditions of the experiment, a wavelength may be chosen where a_s has a valve which results in the cell of solution having a transmittancy near the optimum for concentration determination as discussed in Section 7·27.

7·35 Check of additive absorbancies. In most spectrophotometric work, provided the instrument has fairly good resolution, and provided the solution conditions are carefully chosen, failure of Beer's law is not a major problem. Sometimes Beer's law failures which are reported are caused by the instrument and not by the solution. Thus Vandenbelt *et al.* (*199*) reported that the instrument they were using gave apparent failures of Beer's law in the same direction and at the same transmittancies for several different compounds and concluded that the error was in the instrument. Errors such as these can be avoided by making tests against standard reference filters of known transmittance. It is often possible to select solution conditions in which absorbancies are additive, although under other conditions absorbancies are not additive (*194*). It is often possible to select wavelengths where absorbancies are additive, although at other wavelengths they are not. This is illustrated by formyl violet S4B (Fig. 7·6). It is often possible to have most of the solutions of unknowns analyze within 50 per cent of the concentration of the standard, and Beer's law may be obeyed over this small range even if it deviates over large ranges. It is seldom necessary to correct spectrophotometric data for Beer's law failure when the analytical method has been very carefully designed.

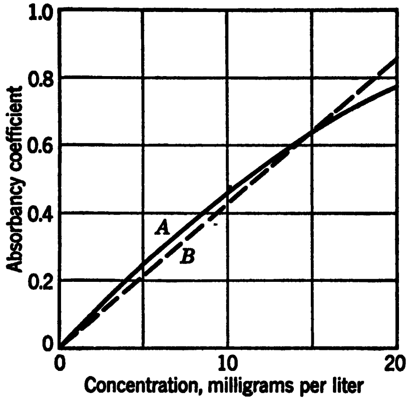

FIG. 7·29. Curves showing check on Beer's law for formyl violet S4B at 588 mμ.

A = observed curve;
B = theoretical curve based on the observed absorbancy coefficient at the standard concentration of 15 mg./l. and the assumption of Beer's law.

The conventional way to check Beer's law is to plot absorbancy for a standard cell length versus concentration and inspect the curve. It is a straight line if Beer's law is followed. Figure 7·29 shows a plot of this type for formyl violet S4B at 588 mμ, where Beer's law fails. Assuming Beer's law to hold, and calculating absorbancies on the basis of the standard solution at a concentration of 15 mg./l., it would be expected that an absorbancy of 0.2 would correspond to a concentration of 4.6. Actually it is found by experiment to correspond to 4.0. This means that Beer's law failure will introduce an error in concentration of 0.6 mg./l. on an absolute basis, or 0.6/4.0 = 15 per cent on a relative basis, unless proper correction is made. If it is desired to assume Beer's law and introduce an error no larger than 0.2 mg./l., it is necessary to adjust the concentration of the unknown so that its absorbancy will fall between 0.66 and 0.60. These values are found by inspecting Fig. 7·29 and determining at what absorbancy above and below the standard value of 0.64 the horizontal separation of curves *A* and *B* exceeds 0.2 unit as measured on the abscissa. If it is desired to assume Beer's law and introduce an error no larger than 1.3 per cent, it is more convenient to plot logarithm of absorbancy versus logarithm of concentration, as is done in Fig. 7·30, and determine where the horizontal separation of curves *A* and *B* exceeds 0.0056 unit, as measured on the abscissa (0.0056 is the logarithm of 1.013). It will be noted that, although curves *A* and *B* of Fig. 7·29 come together at low concentrations, indicating decreas-

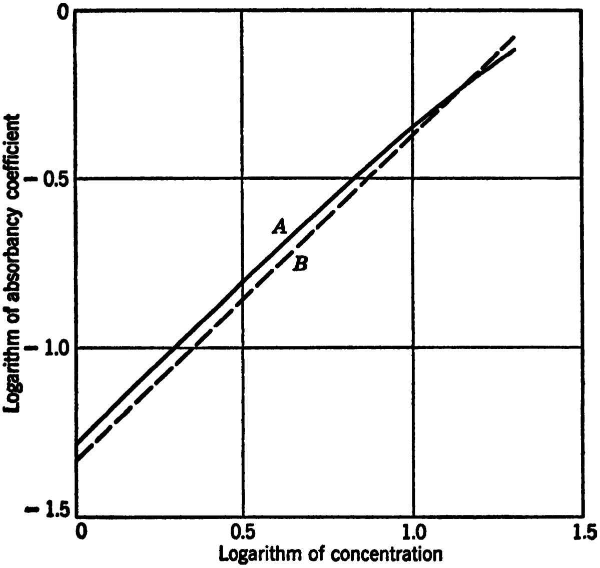

FIG. 7·30. Curves showing check on Beer's law for formyl violet S4B at 588 mμ.
A = observed curve;
B = theoretical curve based on the observed absorbancy coefficient at the standard concentration of 15 mg./l. and the assumption of Beer's law.

ing absolute errors, curves A and B of Fig. 7·30 stay apart, indicating that these errors are large on a relative basis.

7·36 Algebraic method, additive absorbancies. The determination of concentration of a known component at an unknown concentration, when no other absorbing materials are present and with additive absorbancies, depends upon solving equation (7·12):

$$c_a = \frac{(k_s)_{m\lambda_1}}{(a_s)_{a\lambda_1}} \tag{7·12}$$

where c_a = concentration of component a present.

$(k_s)_{m\lambda_1}$ = absorbancy coefficient of the solution of unknown concentration at wavelength λ_1.

$(a_s)_{a\lambda_1}$ = absorbancy index of component a at wavelength λ_1.

It will be recalled that in accordance with equations (7·1)

$$(k_s)_{m\lambda_1} = \frac{\log_{10} [1/(T_s)_{m\lambda_1}]}{b_m} \tag{7·13}$$

$$(a_s)_{a\lambda_1} = \frac{\log_{10} [1/(T_s)_{a\lambda_1}]}{b_\alpha c_\alpha} \tag{7·14}$$

where $(T_s)_{m\lambda_1}$ = transmittancy of the solution of unknown concentration at wavelength λ_1.

b_m = thickness of the cell used in the measurement of the solution of unknown concentration.

$(T_s)_{a\lambda_1}$ = transmittancy of a solution of a at a known concentration c_a.

$b_\alpha c_\alpha$ = thickness of the cell and the concentration used in the measurement of the known solution.

It is necessary to ascertain the value of $(a_s)_{a\lambda_1}$ and put this numerical value in equation (7·12) before any analyses may be made. This is evaluated in accordance with equation (7·14) by measuring a standard material. Whether the test is a self-measurement or color development method, a suitable standard material is usually a "pure" compound. Sometimes the "pure" compound will be impure but will represent the best available material. In such cases it is possible that some samples will analyze over 100 per cent. This is a reflection on the purity of the standard, not on the accuracy of the method.

After equation (7·12) has been established with a numerical value for $(a_s)_{a\lambda_1}$, analyses of unknowns may be carried out by determining the values of $(k_s)_{m\lambda_1}$. This is done by substitution of measured quantities in equation (7·13). These values of $(k_s)_{m\lambda_1}$ are then substituted in equation (7·12).

In some problems, as in determining the grams per liter of Fe^{+++} in a solution, concentration is the desired answer. In some other problems, as in analyzing an organic compound contaminated with a nonabsorbing impurity such as water, purity may be the desired answer. For this purpose, equation (7·12) is modified to

$$P = \frac{100(k_s)_{m\lambda_1}}{c_m(a_s)_{a\lambda_1}} \tag{7·15}$$

where P = percentage purity of the unknown sample.
c_m = concentration at which the unknown sample is prepared for measurement.

Examples of analyses for both concentration and purity are given below.

As an example of determination of concentration, suppose it is desired to calculate the orange II content of a number of solutions of unknown concentration and express the results in grams per liter. This is illustrated by the data of Fig. 7·31 and Table 7·1. The first step is to determine $(a_s)_{a\lambda_1}$ by equation (7·14). A measurement is made of a solution of the standard orange II at a concentration of 0.020 g./l., and it is found that the transmittancy at 485 mμ is 0.190 in a cell of length 1.010 cm. This is curve A of Fig. 7·31. Substituting these values in

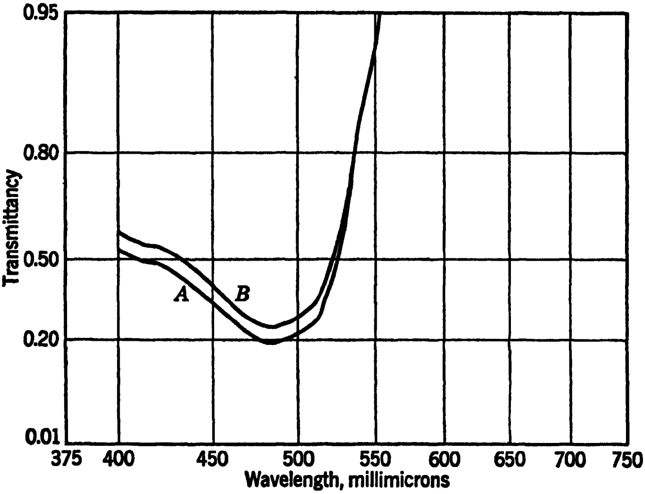

FIG. 7·31. Curves for single-component analysis for orange II.
A = standard;
B = sample of unknown concentration.
Solvent = water.

equation (7·14) gives $(a_s)_{a\lambda_1} = 35.69$. Equation (7·12) then becomes

$$c_a = 0.0271(k_s)_{m\lambda_1} \tag{7·16}$$

Equation (7·16) is the standard formula for the calculation of concentrations of orange II algebraically.

A solution of orange II of unknown concentration is then measured in a 0.998-cm. cell and found to have a transmittancy of 0.250 at 485 mμ. This is shown as curve B in Fig. 7·31. The value of $(k_s)_{m\lambda_1}$ is found by substitution in equation (7·13) to be 0.603. Substituting 0.603 in equation (7·16) gives $c_a = 0.0169$ g./l.

TABLE 7·1 ORANGE II OF FIG. 7·31 AT 485 Mμ

	T_s	a_s	k_s
Standard	0.190	35.69	.....
Sample	0.250	.....	0.603

As an example of determination of purity, suppose that a standard dye powder is available, and also a sample contaminated with water and sodium chloride. Suppose that the standard dye powder was found to have $(a_s)_{a\lambda_1} = 35.69$ by the use of equation (7·14). Equation (7·15) then becomes

$$P = \frac{2.71(k_s)_{m\lambda_1}}{c_m} \tag{7·17}$$

Equation (7·17) is the standard formula for determining the purity of orange II. If a solution made up at 0.020 g./l. has a transmittancy of 0.250 in a 0.998-cm. cell at 485 mμ, equation (7·13) may be used to calculate $(k_s)_{m\lambda_1}$. Substitution of this value, and the value for c_m in equation (7·17), gives $P = 84.5$ per cent.

7·37 Algebraic method, nonadditive absorbancies. If Beer's law fails, and it is inconvenient to measure the solution of the unknown so that its transmittancy falls close enough to the standard to require no correction, a correction may be made mathematically. The simplest method of correction is to prepare a plot of absorbancy coefficient versus concentration, as is done in Fig. 7·29, curve A. The concentration corresponding to the observed absorbancy coefficient is determined by reference to the curve. Such a curve may be called an empirical correlation curve.

A suitable correction may also be made by the method of successive approximations. A plot is made of $(a_s)_{a\lambda_1}$ versus concentration, as illustrated for formyl violet S4B by Fig. 7·32. This plot is determined by measuring standard formyl violet S4B at a number of concentrations, calculating the absorbancy index at each concentration, and making a smooth curve through the points. Suppose that a sample of unknown concentration is measured and found to have a transmittancy of 70 per cent in a 1.000-cm. cell, which means that $(k_s)_{a\lambda_1} = 0.155$. Figure 7·32 shows that the value to be substituted for $(a_s)_{a\lambda_1}$ in equation (7·12) can vary from 39 to 50. It does not matter what value is used first. If 40 is used, equation (7·12) becomes

$$c_a = \frac{(k_s)_{a\lambda_1}}{40} = \frac{0.155}{40} = 0.00387 \text{ g./l.}$$

Referring to Fig. 7·32, it is seen that a concentration of 3.87 mg./l. corresponds to $(a_s)_{a\lambda_1} = 49.8$. Using this value in the second approximation,

$$c_a = \frac{(k_s)_{a\lambda_1}}{49.8} = \frac{0.155}{49.8} = 0.00311 \text{ g./l.}$$

Referring to Fig. 7·32, it is seen that a concentration of 3.11 mg./l. corresponds to $(a_s)_{a\lambda_1} = 50.2$. Using this value in the third approximation gives

$$c_a = \frac{(k_s)_{a\lambda_1}}{50.2} = \frac{0.155}{50.2} = 0.00309 \text{ g./l.}$$

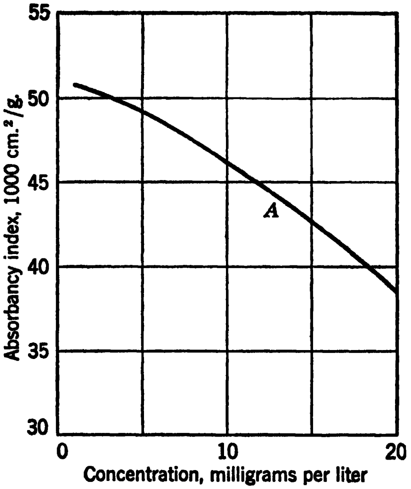

FIG. 7·32. Correction curve for Beer's law failure for formyl violet S4B at 588 mμ, using method of successive approximations.

This is not significantly different from the second approximation and may be accepted as the correct value.

7·38 Log A_s method, additive absorbancies. It was pointed out in Section 7·3 that the vertical height of the absorption curve plotted on a log A_s ordinate is a function of concentration. The log A_s method of determining concentrations from such a plot on transparent paper, using the logarithmic reference scale described in Section 7·3, will now be explained. If the reference logarithmic scale is placed on the plot so that the scale falls along one wavelength and the 1 mark falls at 0.10 transmittancy, absorbancies may be read directly from the scale. The reading on the reference scale at the intersection of a plotted curve with it will be the absorbancy of the corresponding solution. If the unknown happened to be measured in a 1.000-cm. cell, $(A_s)_{m\lambda_1} = (k_s)_{m\lambda_1}$. The intersection of the plotted curve with the reference scale in this position would then read $(k_s)_{m\lambda_1}$, and this value could be substituted in equation (7·16) to calculate c_a. However, if the reference scale is placed so that it is vertically displaced, the displacement being such that 0.0271 falls at 0.10 transmittancy, then the scale reads $0.0271(A_s)_{m\lambda_1}$. In the special case of unknowns measured in a 1.000-cm. cell, $(A_s)_{m\lambda_1} = (k_s)_{m\lambda_1}$, the scale reads $0.0271(k_s)_{m\lambda_1}$, which is the right-hand side of equation (7·16), and the intersection of the plotted curve of the unknown would read c_a directly. Figure 7·33 shows the standard curve for the analysis of samples. The standard curve consists merely of the reference scale placed vertically so that 0.0271 falls at 0.10 transmittancy.

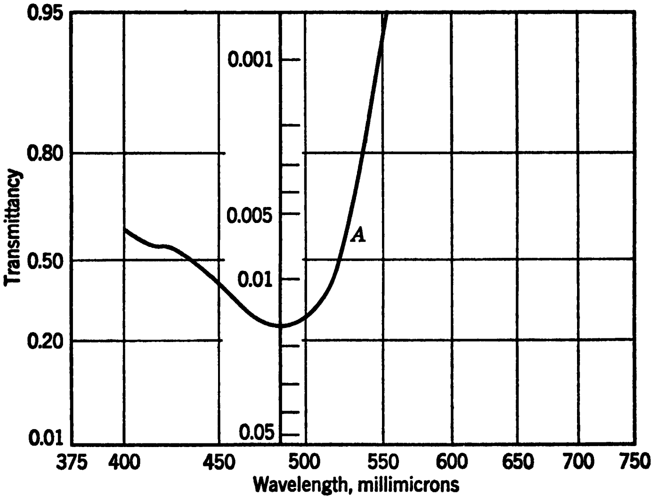

FIG. 7·33. Curve for log A_s method of analysis for one component (orange II). A = solution of unknown concentration adjusted for cell length. Vertical scale is log scale placed so that 0.0271 falls at 0.10 transmittancy.

If the cell used for the measurement of the unknown is 1.000 cm., when the curve is traced on the standard curve plot, the intersection with the reference scale will indicate the concentration. If the cell used is not 1.000 cm., the curve of the unknown may be vertically displaced on the standard curve to correct for this cell-length difference from 1.000. Figure 7·33 illustrates this for the data of the unknown listed in Table 7·1.

Curve B of Fig. 7·31 is traced on Fig. 7·33, but, instead of being at 0.250 transmittancy as it measured, it is traced slightly lower in the position where it would have been if the cell in which it was measured had been 1.000 instead of 0.998 cm. This vertical displacement may be determined with the reference log scale. The curve is traced so that its measured vertical position coincides with 0.998 on the log scale and the adjusted position coincides with 1.000 on the log scale. The intersection of the adjusted curve with the nomographic log scale then gives the concentration directly. It represents a complete solution of equations (7·12) and (7·13). In practice, of course, the curves are not traced on the standard nomograph, but instead they are plotted on transparent paper and superimposed on the standard nomograph. Also, in practice the scale is not drawn on the nomograph, but only one point is indicated. Then the transparent reference scale is placed so that the designated number falls at the indicated point.

It will be noted that in the nomographic method no transmittancies are read to arrive at the concentration. Only one absolute value of transmittance must be known on the nomograph and on the plot of the unknown. These transmittance values must be known so that they may be vertically displaced by an amount corresponding to the cell-length correction. With instruments of the automatically recording type it is convenient to use as this known transmittance the plot of a standard calibration gray filter drawn by the instrument. Use of the filter line drawn automatically by the instrument corrects the data for vertical positioning errors in placing plotting paper on the instrument. If, in addition, a suitable wavelength calibration has been drawn by the instrument on the plot of the unknown and is also included on the standard nomograph, the data may be automatically corrected for horizontal positioning errors by aligning the wavelength calibrations. Since no transmittancies or wavelengths are read from the plotting paper, there is no need for a grid and the curves may be run on blank transparent paper. Merely by positioning the plot of the unknown accurately on the nomograph, and placing the reference scale correctly on top of both, the concentration may be read directly. All corrections have then been made for paper-positioning errors in obtaining the data, and equations (7·12) and (7·13) have been solved.

Although the grid is not used, it is convenient to have a grid so that, in discussing the data, reference may be made to particular portions of the curve. Likewise, although the absorption curve of the standard material is not used in the actual calculation of concentration, it is convenient to have it on the standard curve plot for purposes of qualitative analysis. Before making a quantitative calculation, the curve of the sample is first placed on the standard curve and adjusted vertically so that the curve shapes may be compared. (See Section 7·40, Fig. 7·35.) If the curve shapes are identical, most of the common gross errors have been eliminated. For instance, the material has been correctly labeled as to identity, the solutions have been properly prepared and measured, and there is no contamination with absorbing materials. If the curve shapes do not agree, then the calculation of concentration should not be made. This is one of the great advantages of spectrophotometry over filter photometry. In the latter only a single transmittancy is measured and the concentration calculated. If the calculated concentration is unexpected, one might suspect an error of some kind, such as the solution being prepared at an incorrect *p*H value, but would never know. With spectrophotometric data, the preliminary comparison of curve shape would immediately show any significant error of this kind. Thus any quantitative analysis is really preceded by a qualitative analysis to make sure that the standard curve applies.

When it is desired to determine the purity of a sample, which means solving equation (7·17) nomographically, a standard concentration may be adopted. For the data of Table 7·1 the standard concentration might be 0.020 g./l. Equation (7·17) would then become

$$P = 135.5(k_s)_{m\lambda_1} \tag{7·18}$$

A figure similar to Fig. 7·33 would then be drawn, but with the reference scale reading 135.5 at 0.10 transmittancy. This would be the standard curve for purity analyses. If the sample of unknown purity were not measured at the standard concentration, it could be calculated graphically to the standard concentration. This is done by placing the reference scale on the curve of the unknown so that the reference scale reads the concentration at which the unknown was measured. With the reference scale in this position, a mark is made on the plot opposite the standard concentration as read on the reference scale. The intersection of this mark on the scale of the standard curve is then the purity.

7·39 Log A_s method, nonadditive absorbancies. In the event of Beer's law failure, the standard curve to be used in the graphical analysis has more than one standard point of reference. Enough points are chosen so that, if the curve of the unknown is read against the nearest point, the error will be insignificant. For formyl violet S4B (see Fig. 7·29, curve *A*), the standard curve is shown in Fig. 7·34. The marks labeled 2, 5, 10, and 20, which indicate concentration in milligrams per liter, do not fall at the vertical heights predicted by Beer's law. They fall at empirical heights determined by actual measurement of the standard dye at those concentrations. When the curve of the sample of unknown concentration, curve *B*, is superimposed over the standard plot, it is observed that it falls between reference marks 2 and 5. Placing the logarithmic reference scale at each of these points gives graphical analyses of 0.00308 and 0.00310, respectively. Since the curve is closer to the 2 reference mark, the former value is taken as the correct analysis.

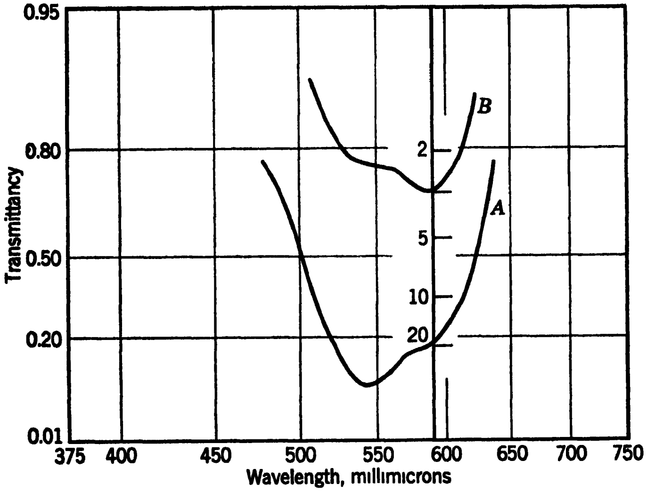

FIG. 7·34. Curves showing correction for Beer's law failure for formyl violet S4B at 588 mμ.

A = standard curve at 20 mg./l.;
B = curve of unknown.

Points numbered at 588 mμ are determined empirically.

7·40 Method of absorbancy coefficient differences. In order to solve for c_a in equation (7·12) it is necessary to determine $(k_s)_{m\lambda_1}$. In the examples which have been considered so far, the only absorbing material present in the solution has been component a, and $(k_s)_{m\lambda_1}$ could be measured directly. If the test solution contains both an unknown absorbing material u and the desired constituent a, the absorbancy coefficient of the test solution will be $[(k_s)_a + (k_s)_u]$. If it is somehow possible to determine $(k_s)_u$, the difference in these two absorbancy coefficients will be $[(k_s)_a + (k_s)_u] - (k_s)_u = (k_s)_a$. This $(k_s)_a$ may be substituted in equation (7·12) to solve for c_a. This makes it possible to solve quantitatively for one component even if another component is present. The various methods available for determining $(k_s)_u$ will be illustrated by the following examples.

As an example of an absorbancy coefficient difference analysis, the work of Johnson (*103*) on vitamin C may be considered. The solutions he worked with contained vitamin C, for which a quantitative determination was desired, and some unknown impurities. Vitamin C can be oxidized to a compound nonabsorbing at 260 mμ. The impurities were unchanged in absorption at 260 mμ by oxidation. The solution was first measured to determine its absorbancy coefficient at 260 mμ. It was then subjected to a chemical oxidation, and finally the absorbancy coefficient again determined at 260 mμ. The first measurement gave the value $[(k_s)_a + (k_s)_u]$, where a represents vitamin C and u represents the nonoxidizable impurities. The measurement of the same solution after oxidation gave the value $(k_s)_u$. Subtracting these two absorbancy coefficients gives $(k_s)_a$, the quantity needed to substitute in equation (7·12) to solve for c_a.

The vitamin C analysis illustrates the total destruction of the absorbing properties of a component at one wavelength which permits the analysis to be made by a method of absorbancy coefficient difference. A similar but reverse chemical process may be illustrated by the determination of arachidonic acid by Brice and Swain (*25*). Arachidonic acid dissolved in alcohol does not absorb at 316 mμ. A first measurement at 316 mμ thus gives $(k_s)_u$, the absorbancy coefficient of other materials present in the sample that do absorb at 316 mμ. The sample of fatty acid is then subjected to an alkali treatment in glycerol, and this converts the arachidonic acid into a conjugated isomeric form that does absorb at 316 mμ. This is then measured and yields the value of $[(k_s)_a + (k_s)_u]$, where a represents a conjugated acid proportional in concentration to the arachidonic acid originally present. By taking the difference in these two absorbancy coefficients, the quantity $(k_s)_a$ is obtained, and this may be used in equation (7·12) to solve for c_a.

Where a color is developed, which is very common in analyses for metals in solution, the process of development may yield some color with a blank. These systems may be analyzed by the method of absorbancy differences. A blank is measured, the sample is measured, and the absorbancy difference taken as $(k_s)_a$. It is assumed that $(k_s)_u$ is the same in both sample and blank.

The process of measuring a sample versus a blank is in itself a method of absorbancy coefficient differences in which the spectrophotometer performs the subtraction. Spectrophotometers measure the ratio of the flux of the sample beam to the flux of the reference beam. In measuring a sample versus a solvent, spectrophotometers determine what is known as transmittancy. In measuring a sample versus a blank, which is of interest in the present discussion, they measure the ratio of transmittancies:

$$R_{(T_s)} = \frac{(T_s)_a}{(T_s)_u} \qquad (7\cdot 19)$$

where $R_{(T_s)}$ = the ratio of two transmittancies.

$(T_s)_a$ = the transmittancy of the sample containing both a and impurities u.

$(T_s)_u$ = the transmittancy of the blank containing only impurities u.

If all cells have a length of 1 cm.,

$$(T_s)_a = 10^{-(k_s)_a-(k_s)_u} \tag{7·20}$$

$$(T_s)_u = 10^{-(k_s)_u} \tag{7·21}$$

and, substituting equation (7·20) and (7·21) in equation (7·19),

$$R_{(T_s)} = 10^{-(k_s)_a-(k_s)_u+(k_s)_u} = 10^{-(k_s)_a} \tag{7·22}$$

Equation (7·22) signifies that the ratio of transmittancies, which is the number read from the spectrophotometer scale, is really the antilog of an absorbancy coefficient difference.

The method of absorbancy coefficient differences is still more useful. In the above examples the absorbancy coefficient of the desired component is zero in at least one measurement. With vitamin C it is zero for oxidized solution, with arachidonic acid it is zero for the untreated solution, and with the color-development tests it is zero for the blank. It does not need to be zero in either measurement.

Bromothymol blue at 450 mμ has a value of $a_s = 24.5$ at pH 5. When the pH is changed to 11, $a_s = 3.2$. If $(k_s)_5$ is the absorbancy coefficient at 450 mμ of a solution of bromothymol blue of concentration c_a at a pH of 5, and $(k_s)_{11}$ is the absorbancy coefficient of the solution when the pH has been changed to 11, the ratio of absorbancy coefficient differences to absorbancy index differences is

$$\frac{(k_s)_5 - (k_s)_{11}}{(a_s)_5 - (a_s)_{11}} = \frac{c_a(a_s)_5 - c_a(a_s)_{11}}{(a_s)_5 - (a_s)_{11}} = \frac{c_a[(a_s)_5 - (a_s)_{11}]}{(a_s)_5 - (a_s)_{11}} = c_a \tag{7·23}$$

Equation (7·23) shows that the ratio of absorbancy coefficient differences to absorbancy index differences is a measure of concentration.

Suppose that, mixed with the bromothymol blue in the above example, there is an additional material x, having an absorption, and that this foreign material is not subject to change with pH. The observed absorbancy coefficients of the samples will then be $(k_s)_5 + (k_s)_x$ and $(k_s)_{11} + (k_s)_x$ instead of merely $(k_s)_5$ and $(k_s)_{11}$. This will not affect the calculation of c_a, for in taking the difference one $(k_s)_x$ value cancels the other.

The conventional method of determining the concentration of bromothymol blue would be to measure the solution of *p*H 5 and substitute in the equation

$$c_a = \frac{(k_s)_5}{(a_s)_5} \tag{7·24}$$

If a *p*H-insensitive material having absorption of $(k_s)_x$ is present, the concentration calculated with equation (7·24) will be wrong. Measurements at *p*H's of 5 and 11 and use of equation (7·23) will give the right answer.

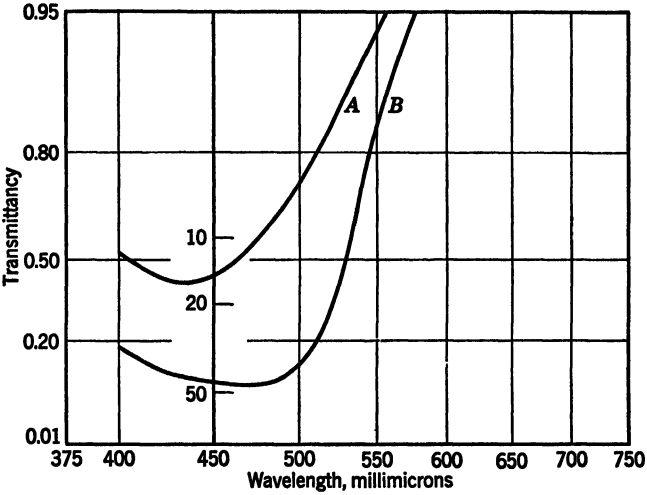

FIG. 7·35. Curves for analysis for bromothymol blue contaminated by the absorbing impurity for orange II.

A = standard curve of bromothymol blue, *p*H 5, 15 mg./l.;
B = curve of mixture of 25 mg./l. bromothymol blue and 20 mg./l. orange II, *p*H 5.

Numbers at 450 mμ indicate reference scale position for concentration evaluation.

As a specific example of the use of absorbancy coefficient differences, consider the analysis of a mixture of 20 mg./l. of orange II (see Fig. 7·33) and 25 mg./l. of bromothymol blue (see Fig. 7·5) for amount of blue present. An attempted conventional analysis is illustrated by Fig. 7·35. Curve *A* is the standard curve of bromothymol blue at a *p*H of 5 and at a concentration of 15 mg./l. When the reference scale is correctly placed to read concentrations, as explained in Section 7·38, it falls as shown in Fig. 7·35 by the scale numbers at 450 mμ. Curve *B* is the absorption curve of the solution, which is a mixture of 20 mg./l.

orange and 25 mg./l. blue. Before using the reference scale to read concentration, curve *B* of the unknown is raised vertically and compared with curve *A* for shape. Since it does not agree in shape, curve *B* is not solely bromothymol blue at a *p*H of 5, the reference scale does not apply, and concentrations may not be read from it. The intersection is found to be at 45 mg./l., which differs from the known valuc of

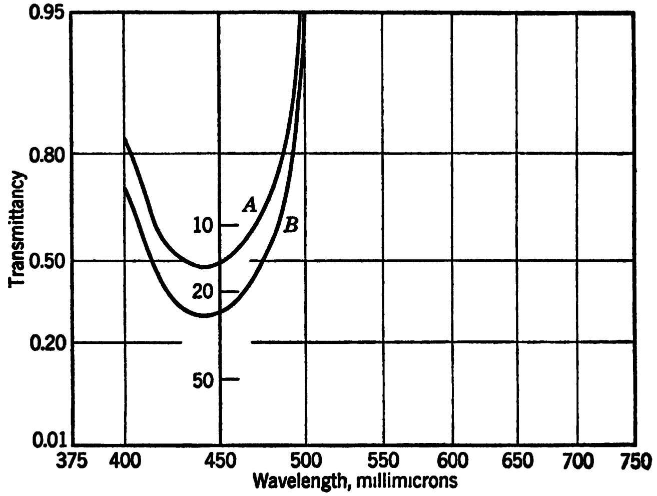

FIG. 7·36. Curves for analysis by absorbancy coefficient difference for bromothymol blue mixed with orange II.

A = standard curve of bromothymol blue, ratio of transmittancies at *p*H 5 to *p*H 11, concentration 15 mg./l.;
B = test curve of mixture of bromothymol blue at 25 mg./l. and orange II at 20 mg./l., ratio of transmittancies at *p*H 5 to *p*H 11.

Numbers at 450 mμ indicate reference scale position for concentration evaluation in grams per liter.

25 mg./l. The conventional analysis would therefore be erroneous because of the presence of the extraneous absorbing material.

Figure 7·36 illustrates the above analysis carried out successfully by the method of absorbancy coefficient differences. Curve *A* of Fig. 7·36 is a ratio of transmittancies curve. It is drawn by comparing a sample beam and a reference beam, when a solution of bromothymol blue at a *p*H of 5 and a concentration of 15 mg./l. is placed in the sample beam, and a solution of the blue at the same concentration but at a *p*H of 11 is placed in the reference beam in a similar cell. The difference in the absorbancy indexes of the bromothymol blue at *p*H values of 5 and 11 is 21.3. Accordingly, the concentration scale is placed on Fig. 7·36 so that the reciprocal of 21.3 falls at the 0.10 transmittancy mark. Curve *A* and the reference scale constitute the standard curve for absorbancy coefficient difference measurements. The test solution, containing 20 mg./l. orange and 25 mg./l. blue, is now measured by placing one solution at a *p*H of 5 in the sample beam and the other solution at a *p*H of 11 in the reference beam. The ratio of transmittancies is plotted as curve *B*. Curve *B* is vertically adjusted to compare its shape with the standard, curve *A*. The fact that the shapes agree means that the orange is not subject to a *p*H effect and that the blue underwent the expected *p*H change. The intersection of curve *B* with the reference scale is therefore properly a measure of concentration. It is observed to be 25 mg./l., the correct answer.

The analyses described above are in a sense two-component analyses in which only one component is determined. In many practical cases the second component is unknown, being perhaps a mixture of unimportant impurities, and is not determined for this reason. If the transmittancies are independently measured, and the ratios calculated, there is justification for calling these methods two-component analyses. It may be noted that in these cases of absorbancy coefficient differences the data can also be used to evaluate the second component, its concentration being proportional to $(k_s)_u$.

7·41 Two components. The two-component analysis in its general form consists of determining the absolute amount of each of two absorbing components present in a mixture which may also contain nonabsorbing components. If the absorption curves of the individual pure components are known, it is possible to determine both components by working at two wavelengths (*203*). It is also possible to work at one wavelength and employ two different solution conditions, as discussed in Section 7·48.

7·42 Choice of wavelengths. Additive absorbancies mean that

$$\begin{aligned} c_a(a_s)_{a\lambda_1} + c_d(a_s)_{d\lambda_1} &= (k_s)_{m\lambda_1} \\ c_a(a_s)_{a\lambda_2} + c_d(a_s)_{d\lambda_2} &= (k_s)_{m\lambda_2} \end{aligned} \qquad (7\cdot25)$$

Solving equations (7·25) simultaneously gives

$$c_a = \frac{\dfrac{(k_s)_{m\lambda_1}}{(a_s)_{d\lambda_1}} - \dfrac{(k_s)_{m\lambda_2}}{(a_s)_{d\lambda_2}}}{\dfrac{(a_s)_{a\lambda_1}}{(a_s)_{d\lambda_1}} - \dfrac{(a_s)_{a\lambda_2}}{(a_s)_{d\lambda_2}}} \qquad c_d = \frac{\dfrac{(k_s)_{m\lambda_1}}{(a_s)_{a\lambda_1}} - \dfrac{(k_s)_{m\lambda_2}}{(a_s)_{a\lambda_2}}}{\dfrac{(a_s)_{d\lambda_1}}{(a_s)_{a\lambda_1}} - \dfrac{(a_s)_{d\lambda_2}}{(a_s)_{a\lambda_2}}} \qquad (7\cdot26)$$

Ordinarily it is desired to calculate the amount of *a* present, c_a, with the greatest percentage accuracy. If Δc_a is the absolute error in the calculation of c_a, it is desired to calculate c_a under conditions such that $\Delta c_a/c_a$ is a minimum. The accuracy of the analysis will depend in part upon the choice of the two wavelengths. Let it be assumed that all absorbancy coefficients may be calculated with the same percentage accuracy. It was shown in Section 7·27 that transmittancies between 0.10 and 0.50 give the more accurate values, at least with some instruments; but if an absorbancy coefficient is small, the solution may be measured in a thick cell or in a more concentrated solution to adjust the transmittancy to the optimum range. Also, it was pointed out in Section 7·34 that all absorbancy coefficients are not calculable with the same accuracy, for the reasons given; but for the purpose of choosing the optimum two wavelengths for solving equation (7·26), let this assumption be made.

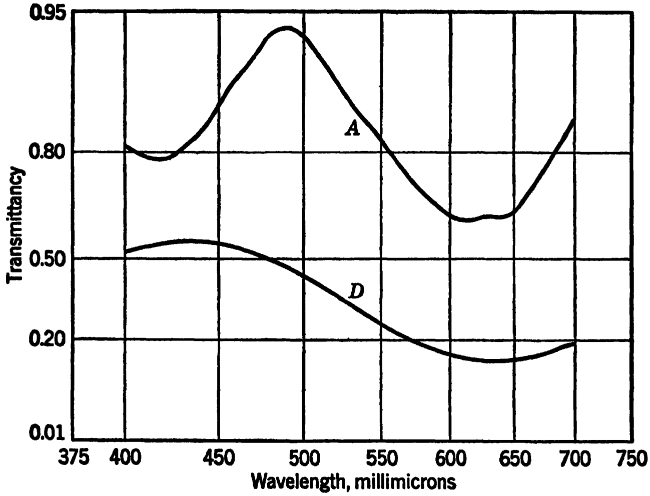

FIG. 7·37. Curves illustrating choice of wavelengths for two-component analysis. Curves *A* and *D* represent two components of a mixture that is to be analyzed.

A = alizarin cyanine green (C.I. 1078) in water;
D = hypothetical impurity.

It can be shown mathematically that the conditions which give the most accurate percentage estimate of c_a, with the above assumption, are those at which $(a_s)_{a\lambda_1}/(a_s)_{d\lambda_1}$ and $(a_s)_{d\lambda_2}/(a_s)_{a\lambda_2}$ are maxima (*13*).

This same calculation shows that the condition for *minimum* accuracy is to have $(a_s)_{a\lambda_1}/(a_s)_{d\lambda_1} = (a_s)_{a\lambda_2}/(a_s)_{d\lambda_2}$. Qualitatively this criterion of accuracy is evident from a consideration of equation (7·26). Because c_a is a constant in any one problem, the numerator and denominator are always in the same ratio, and thus it is necessary to deal only with the denominator in this qualitative consideration of accuracy. If $(a_s)_{a\lambda_1}/(a_s)_{d\lambda_1}$ and $(a_s)_{a\lambda_2}/(a_s)_{d\lambda_2}$ are of similar magnitude, a slight error in the absolute value of either is a large percentage of the difference. A maximum difference between $(a_s)_{a\lambda_1}/(a_s)_{d\lambda_1}$ and $(a_s)_{a\lambda_2}/(a_s)_{d\lambda_2}$ means that an error in the absolute value of either will be a relatively small percentage of the difference and c_a will be determined with most accuracy.

It is possible to draw a graph of $(a_s)_a/(a_s)_d$ and then choose the maximum and minimum of this plot as the best two wavelengths.

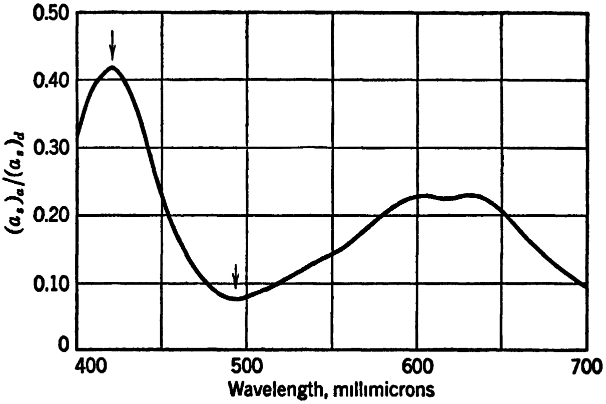

FIG. 7·38. Curve showing optimum wavelengths for two-component analysis. (Components A and D of Fig. 7·37.)

Figure 7·37 shows the absorption curves of two compounds that are to be determined in the presence of each other. Figure 7·38 shows a plot of the ratio of absorbancy indexes of the two compounds of Fig. 7·37. It is evident from Fig. 7·38 that in the wavelength range covered by the instrument the maximum and minimum ratios fall at 420 and 492 mμ.

If the absorption data are plotted on transparent paper and with an ordinate of log A_s, a graphical method may be used to determine the two wavelengths very rapidly. If $(a_s)_a/(a_s)_d$ is to be a maximum, then log $(a_s)_a$ − log $(a_s)_d$ is to be a maximum. Plots of log $(A_s)_a$ are plots of log $(a_s)_a$ with an arbitrary constant vertical displacement that does not matter. Hence, the problem is to find the wavelength where the two plots on a log A_s ordinate are separated vertically by the greatest linear distance. If the absorption curve of component a is not wholly above the absorption curve of component d, the plotting paper should be vertically displaced so that this is true. Then the transparent plot-

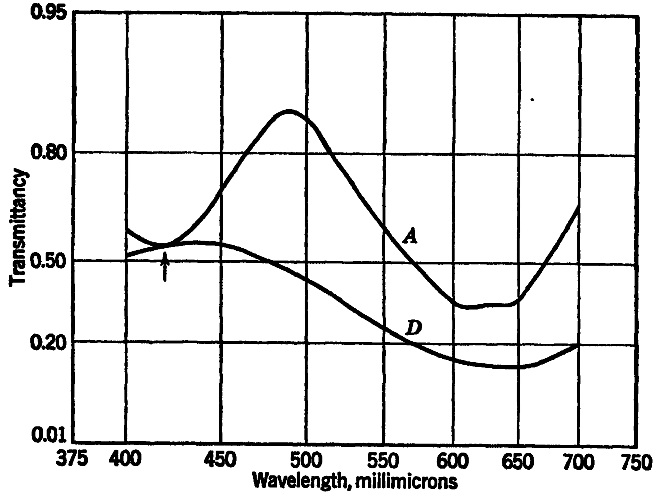

FIG. 7·39. Curves illustrating choice of wavelength for two-component analysis. 420 mμ is found to be the wavelength of maximum $(a_s)_a/(a_s)_d$.

A = alizarin cyanine green (C.I. 1078) in water;
D = hypothetical impurity.

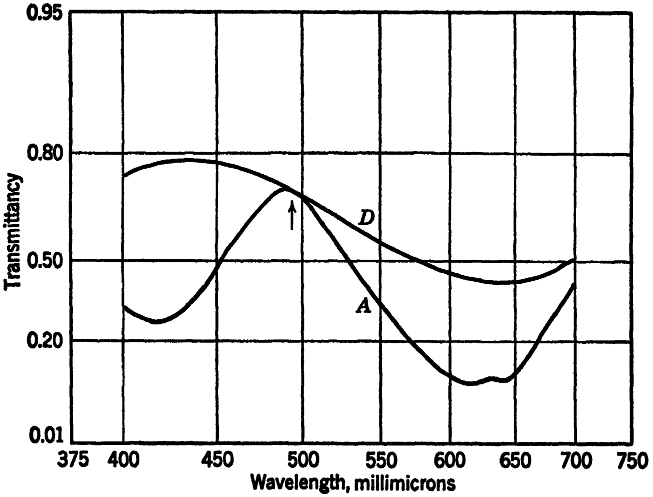

FIG. 7·40. Curves illustrating choice of wavelength for two-component analysis. 492 mμ is found to be the wavelength of minimum $(a_s)_a/(a_s)_d$.

A = alizarin cyanine green (C.I. 1078) in water;
D = hypothetical impurity.

ting paper with the curve of *a* is lowered relative to *d* until the two curves first meet. The point at which they first meet is wavelength λ_1. This is illustrated by Fig. 7·39, where the curve of *a* meets the curve of *d* at 420 mμ. The process is then repeated, but with the curve of component *d* first raised vertically until it is entirely above the curve of *a*, then lowered to the first point of contact. This is illustrated by Fig. 7·40, where the second wavelength of analysis is found for the compounds of Fig. 7·37 to be 492 mμ.

The method described above will give the optimum two wavelengths for analysis from the mathematical viewpoint, but in addition all the factors considered in Section 7·34 apply and the optimum wavelengths first found are modified accordingly.

7·43 Check of additive absorbancies. Even if the individual components follow Beer's law by themselves, it may not be assumed that mixtures of the two have additive absorbancies. Known mixtures in the range of concentrations of interest should be prepared and the additivity of absorbancy coefficients checked. The two dyes chrysophenine and diamine sky blue FF obey Beer's law over a fairly large range when tested individually. Figure 7·41 shows the straight line *A* which would be found for the relationship of absorbancy coefficients to relative proportion of dyes if absorbancy coefficients were additive, and also curve *B* which is the observed relationship determined empirically. This is a component interaction effect which was pointed out in Section 7·18 and which can sometimes be eliminated by change of solvent.

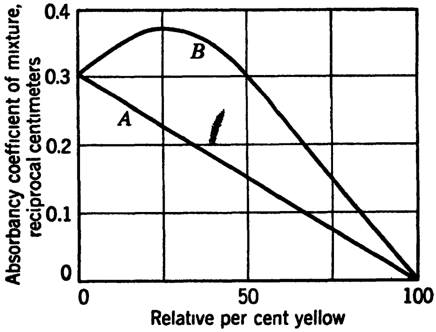

FIG. 7·41. Curves showing check of additive absorbancies. Chrysophenine 50 per cent and diamine sky blue FF; total concentration = 37.5 mg./l.; 675 mμ.

A = straight line relation expected with additive absorbancies;
B = observed relation.

The empirical checks should cover not only the range of relative proportions of interest, but also the range of total concentrations of interest.

7·44 Algebraic method, additive absorbancies. Any analysis for two components by spectrophotometric methods depends fundamentally upon the solution of equations (7·25). It may appear that there are several distinct cases, as, for instance, whether or not both components absorb at both wavelengths; but such special cases are simplifications of the general case. Solving equations (7·25) and expressing the result in the form of determinants,

$$c_a = \frac{\begin{vmatrix} (k_s)_{m\lambda_1} & (a_s)_{d\lambda_1} \\ (k_s)_{m\lambda_2} & (a_s)_{d\lambda_2} \end{vmatrix}}{\begin{vmatrix} (a_s)_{a\lambda_1} & (a_s)_{d\lambda_1} \\ (a_s)_{a\lambda_2} & (a_s)_{d\lambda_2} \end{vmatrix}} \qquad c_d = \frac{\begin{vmatrix} (a_s)_{a\lambda_1} & (k_s)_{m\lambda_1} \\ (a_s)_{a\lambda_2} & (k_s)_{m\lambda_2} \end{vmatrix}}{\begin{vmatrix} (a_s)_{a\lambda_1} & (a_s)_{d\lambda_1} \\ (a_s)_{a\lambda_2} & (a_s)_{d\lambda_2} \end{vmatrix}} \tag{7·27}$$

Letting $|D| = \begin{vmatrix} (a_s)_{a\lambda_1} & (a_s)_{d\lambda_1} \\ (a_s)_{a\lambda_2} & (a_s)_{d\lambda_2} \end{vmatrix}$,

$$\begin{aligned} c_a &= \frac{(a_s)_{d\lambda_2}}{|D|}(k_s)_{m\lambda_1} - \frac{(a_s)_{d\lambda_1}}{|D|}(k_s)_{m\lambda_2} \\ c_d &= \frac{(a_s)_{a\lambda_1}}{|D|}(k_s)_{m\lambda_2} - \frac{(a_s)_{a\lambda_2}}{|D|}(k_s)_{m\lambda_1} \end{aligned} \tag{7·28}$$

Equations (7·25) and (7·28) are equivalent. One set is obtained from the other by linear transformation. If an analysis is to be carried out only once, it does not matter which is used. But if several analyses are to be carried out, and in particular if a standard method of analysis is to be set up in a commercial laboratory, equations (7·28) are much to be preferred as they lead to less computation. It will be noted that the coefficients of equations (7·28), namely, $(a_s)_{d\lambda_2}/|D| \cdots$, are constants which need be calculated only once. Once these constants are evaluated, a routine analysis which is based upon experimentally determined values of $(k_s)_{m\lambda_1}$ and $(k_s)_{m\lambda_2}$ consists merely in subtracting two products, a numerical process which is more easily carried out than the solution of simultaneous equations as required if equations (7·25) are used.

The constants $(a_s)_{d\lambda_2}/|D| \cdots$ may be determined in one of three ways. First, the method of direct substitution may be used. The values of components *a* and *d* are determined at two wavelengths, λ_1 and λ_2, and substituted in the expressions $(a_s)_{d\lambda_2}/|D| \cdots$. This is probably the simplest method for two-component systems.

Second, a modification of Crout's method (*56*) may be used. This requires a modern calculating machine of the type that will perform a division on the sum of products as one calculating process. Actually, this method is more useful in cases of four or five components but will be introduced here for pedagogic reasons. Crout's method of solution of simultaneous equations is a method whereby equations (7·25) may be solved for c_a and c_d if all the k_s values are given. It will not be presented here because it is given in detail in the reference and also in the instructions for operation of calculating machines with which the method can be used. A slight modification is necessary to calculate the coefficients of equations (7·28). The values of $(a_s)_a$ and $(a_s)_d$ in equation (7·25) are fixed by the components. However, the values of $(k_s)_{m\lambda_1}$ and $(k_s)_{m\lambda_2}$ will vary from test to test. The modification is to assume that $(k_s)_{m\lambda_1} = 1$ and $(k_s)_{m\lambda_2} = 0$ and then solve for c_a and c_d by Crout's method. It will be noted in equations (7·28) that, if $(k_s)_{m\lambda_1} = 1$ and $(k_s)_{m\lambda_2} = 0$, then $c_a = (a_s)_{d\lambda_2}/|D|$, which is one of the constants desired, and $c_d = -(a_s)_{a\lambda_2}/|D|$, which is another of the desired constants. Next, another hypothetical case is assumed in which $(k_s)_{m\lambda_1} = 0$ and $(k_s)_{m\lambda_2} = 1$. Solving equations (7·25) for these values by Crout's method gives $c_a = -(a_s)_{d\lambda_1}/|D|$ and $c_d = (a_s)_{a\lambda_1}/|D|$, which are the other two desired constants. Thus, using a modern calculating machine, and using Crout's method to solve the simultaneous equations (7·25) for special values of $(k_s)_{m\lambda_1}$ and $(k_s)_{m\lambda_2}$, the coefficients of equations (7·28) may be calculated.

Third, if an electrical computing machine is available, the constants may be calculated with it. At least two such electrical instruments are available (*14, 15, 151, 171*). They are particularly useful when multicomponent analyses of six up to eleven components are to be made. However, this method is included at this point for pedagogic reasons. The details of operation of these instruments are not discussed here, as they are included in the operating instructions for the instruments, which solve by successive approximations equations of the type of equations (7·25). Again, a modification is needed in using these instruments to determine the constants of equations (7·28), which consists in arbitrarily letting $(k_s)_{m\lambda_1} = 1$ and $(k_s)_{m\lambda_2} = 0$ and solving for two of the constants, followed by letting $(k_s)_{m\lambda_1} = 0$ and $(k_s)_{m\lambda_2} = 1$ and solving for the other two.

Regardless of whether calculations are made by slide rule, calculating machine, or electrical computing instrument, equations (7·28) are a better form with which to work if many analyses are to be made. With known constants of equations (7·28), in the case of the slide rule, two multiplications may be made and the answers written down and added to solve for c_a. This is easier than solving simultaneous equations. With the modern calculating machine, the products may be summed directly. This is easier than using Crout's method to solve equations (7·25). With the electrical computing instruments, c_a may be determined directly and exactly from equations (7·28) without going through a series of successive approximations which are necessary when solving equations (7·25).

As a specific example of a problem wherein both components absorb at both wavelengths, consider the data of Fig. 7·42. Suppose that diamine violet N is frequently found with diamine green B in amounts

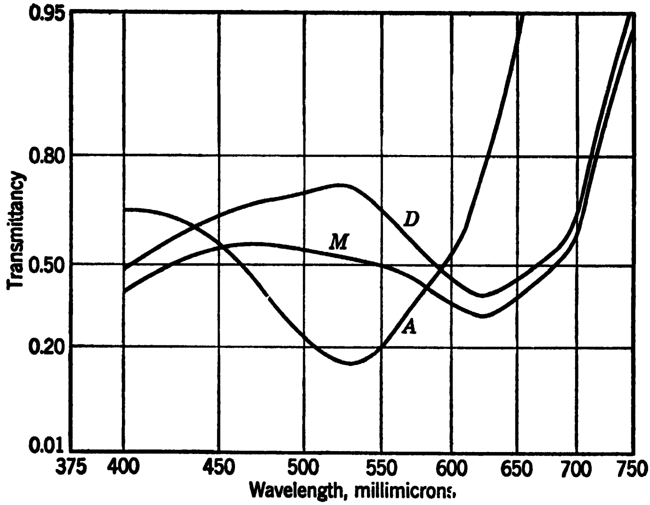

Fig. 7·42. Curves illustrating two-component analysis by algebraic method. Both components absorb at both wavelengths (528 and 625 mμ).

A = diamine violet *N*;
D = diamine green *B*;
M = mix of *A* and *D* of unknown total concentration.

Solvent = water.

ranging from 0 to 25 per cent. The optimum wavelengths for analysis have been found by the method outlined in Section 7·42, and the wavelengths found best by this method have been altered so as to make measurements on a part of the curve which is more nearly horizontal and thus avoid wavelength errors. The chosen wavelengths are 528 and 625 mμ. In order to set up a standard analysis to be used many times over a period of years, equations (7·28) are chosen for purposes of calculation. The observed transmittancies of the two standards prepared at a concentration of 0.040 g./l. and measured in a 0.970-cm. cell are listed in Table 7·2. From these, the absorbancy indexes have been calculated (by equation 7·14) and are also listed in Table 7·2. One of the unknowns was a solution measured in a 1.020-cm. cell. The observed transmittancies of this mix and the calculated absorbancy coefficients of the mix are also listed in Table 7.2.

TABLE 7·2 TWO-COMPONENT ANALYSIS OF FIG. 7·42

	T_s		a_s		k_s	
	528 mμ	625 mμ	528 mμ	625 mμ	528 mμ	625 mμ
Violet	0.150	0.790	21.235	2.638	...	.
Green	0.735	0.395	3.446	10.397	..	.
Mix	0.520	0.317	.	.	0.2784	0.4891

Substituting the values of a_s of Table 7·2 in equations (7·28) gives

$$\begin{aligned} c_a &= 0.04912(k_s)_{m\lambda_1} - 0.01628(k_s)_{m\lambda_2} \\ c_d &= 0.10031(k_s)_{m\lambda_2} - 0.01246(k_s)_{m\lambda_1} \end{aligned} \qquad (7\cdot 29)$$

Equations (7·29) are now available for rapid calculation of mixes. They are the standard equations which are used in the algebraic solution for the amounts of each component present. As an example of their use, an analysis may be made of the unknown mix having the properties tabulated in Table 7·2. Substitution of these values in equations (7·29) gives

$$c_a = 0.04912 \times 0.2784 - 0.01628 \times 0.4891 = 0.0057 \text{ g./l.}$$

of diamine violet N, and

$$c_d = 0.10031 \times 0.4891 - 0.01246 \times 0.2784 = 0.0456 \text{ g./l.}$$

of diamine green B.

Equations (7·29) may be written in the form of equations (7·30) for cases where it is desired to have the results expressed relative to the standards.

$$\begin{aligned} \frac{c_a}{c_m} &= \frac{0.04912(k_s)_{m\lambda_1}}{c_m} - \frac{0.01628(k_s)_{m\lambda_2}}{c_m} \\ \frac{c_d}{c_m} &= \frac{0.10031(k_s)_{m\lambda_2}}{c_m} - \frac{0.01246(k_s)_{m\lambda_1}}{c_m} \end{aligned} \qquad (7\cdot 30)$$

Suppose that an unknown were a mixed dye powder made up at a concentration of 0.100 g./l. and having transmittancies of 0.520 and 0.317 at 528 and 625 mμ in a 1.020-cm. cell. The calculated $(k_s)_{m\lambda_1}/c_m$ and

$(k_s)_{m\lambda_2}/c_m$ would be 2.784 and 4.891 respectively, giving in equations (7·30).

$$\frac{c_a}{c_m} = 0.04912 \times 2.784 - 0.01628 \times 4.891 = 0.057$$

$$\frac{c_d}{c_m} = 0.10031 \times 4.891 - 0.01246 \times 2.784 = 0.456$$

The mix therefore consists of 5.7 per cent of standard diamine violet N, 45.6 per cent of standard diamine green B, and a remainder, to make the weight up to 100 per cent of 48.7 per cent colorless diluent.

A problem of frequent occurrence is to have a mixture of two components with absorption characteristics such that both components absorb at one wavelength but only one absorbs at the second. Equations (7·28) apply, as they do to all two-component systems. However, since $(a_s)_{a\lambda_2} = 0$, considerable simplification is introduced. It will be found that equations (7·28) reduce to

$$\begin{aligned} c_a &= \frac{1}{(a_s)_{a\lambda_1}}(k_s)_{m\lambda_1} - (a_s)_{d\lambda_1} \cdot \frac{(k_s)_{m\lambda_2}}{(a_s)_{a\lambda_1}(a_s)_{d\lambda_2}} \\ c_d &= \frac{1}{(a_s)_{d\lambda_2}}(k_s)_{m\lambda_2} \end{aligned} \qquad (7\cdot31)$$

A wavelength at which only one component absorbs, thus making equations (7·31) apply, will be selected by the method of Section 7·42, if such a wavelength exists.

In such cases, the literature frequently refers to the process of analyzing for one component where it alone absorbs, then correcting the absorption at the other wavelength for absorption of the first component, and analyzing for the second component with the residual absorption (*178*). This amounts to writing equations (7·31) in the form

$$\begin{aligned} c_d &= \frac{(k_s)_{m\lambda_2}}{(a_s)_{d\lambda_2}} \\ c_a &= \frac{(k_s)_{m\lambda_1} - (a_s)_{d\lambda_1}c_d}{(a_s)_{a\lambda_1}} \end{aligned} \qquad (7\cdot32)$$

Although equations (7·32) are perfectly correct, they require that c_d be determined first, regardless of whether it is desired or not. They do not simplify the calculation. There seems to be little reason to deviate from the standard form of equations (7·28) as simplified in this case to equations (7·31).

As a specific example of a problem wherein both components absorb at one wavelength but only one absorbs at the other, consider a mixture of xylene light yellow and orange II analyzed at wavelengths of 410 and 510 mμ, as illustrated in Fig. 7·43. In order to set up a standard method to be used many times, equations (7·31), which are equations (7·28) in the simplified form for this case where $(a_s)_{a\lambda_2} = 0$, are utilized. The observed transmittancies of the two standard dyes prepared at a concentration of 0.020 g./l. and measured in a 1.000-cm. cell are listed in Table 7·3. From these data the absorbancy indexes have been calculated and are also listed in Table 7·3. Later a solution mixture of unknown proportions was measured in a 0.970-cm. cell. The observed transmittancies and calculated absorbancy coefficients are given in Table 7·3.

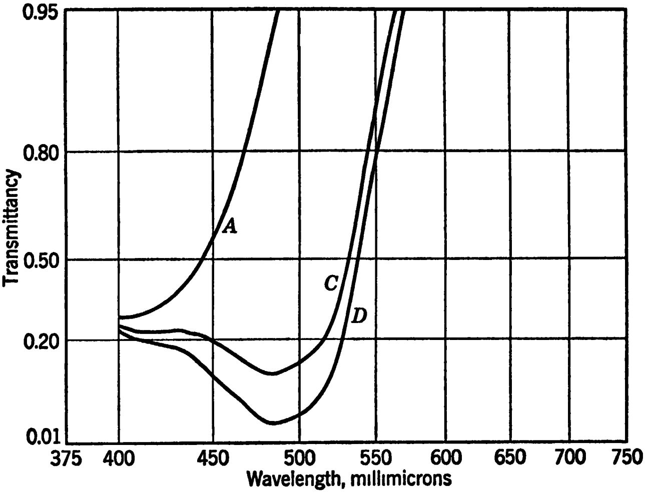

FIG. 7·43. Curves illustrating two-component analysis by algebraic method. Both components absorb at 410 mμ, only one at 510 mμ.

A = xylene light yellow;
D = orange II;
C = mix of A and D of unknown total concentration.

Solvent = water.

TABLE 7·3 TWO-COMPONENT ANALYSIS OF FIG. 7·43

	T_s		a_s		k_s	
	410 mμ	510 mμ	410 mμ	510 mμ	410 mμ	510 mμ
Yellow	0.290	1.000	26.88	0.000	.....	.....
Orange	0.205	0.045	34.41	67.34	.....	.....
Mix	0.232	0.168	.....	......	0.654	0.799

Substitution of the values of Table 7·3 in equations (7·31) gives

$$\begin{aligned} c_a &= 0.03720(k_s)_{m\lambda_1} - 0.01901(k_s)_{m\lambda_2} \\ c_d &= 0.01485(k_s)_{m\lambda_2} \end{aligned} \qquad (7\cdot 33)$$

Equations (7·33) are available for rapid computation of subsequent unknowns. As an example of their use, the data for the solution of unknown concentration listed in Table 7·3 may be substituted in them to solve for the amounts of each component.

$$c_a = 0.03720 \times 0.654 - 0.01901 \times 0.799 = 0.0091 \text{ g./l.}$$

$$c_d = 0.01485 \times 0.799 = 0.0119 \text{ g./l.}$$

The solution, therefore, contains 0.0091 g./l. of xylene light yellow and 0.0119 g./l. of orange II.

To repeat what was said above, there may appear to be many special cases of two-component analyses, but fundamentally there is only one case. This one is represented by equations (7·28), and the special cases are only simplifications of this one general pair of equations. A further simplification is discussed in Section 7·48 and illustrated there by the determination of ascorbic acid.

7·45 Algebraic method, nonadditive absorbancies. Correction for nonadditivity of absorbancy coefficients is more difficult for multicomponent, than for single-component, determinations. With two components there are two factors which may cause additivity to fail. One is that a certain material may not follow Beer's law when it alone is made up in solution. This same failure will probably occur when it is present in mixtures. The failure is illustrated in Fig. 7·29. The second factor is that the absorbancy coefficient of the mixture of the two may not be the sum of the absorbancy coefficients of the two alone. This failure is illustrated in Fig. 7·41. Two methods of analysis for nonadditive absorbancy systems are presented below: empirical correlation and incremental analysis.

A family of correction curves is needed in an empirical correlation over the entire range of possible mixtures because both the above factors may vary with total concentration. For any one concentration a simple way to set up empirical correlation curves is illustrated with the data of Table 7·4. Columns 1 and 2 give the known concentrations of chrysophenine and diamine sky blue FF, respectively. Columns 3 and 4 give the observed absorbancy coefficients at wavelengths of 430 and 650 mμ, respectively. Columns 5 and 6 give the calculated concentrations of yellow and blue, respectively, assuming additive absorbancies and using equations (7·28). Column 7 gives the calculated percentage of yellow relative to total dye. Column 8 gives the calculated total dye concentration. The empirical correction curve for errors in total concentration is shown in Fig. 7·44, which is a plot of the sum of columns 5 and 6, Table 7·4, divided by the sum of columns 1 and 2, versus column 7. The empirical correlation curve for errors in relative proportion is shown in Fig. 7·45, which is a plot of the data of column 1, Table 7·4, versus the data of column 7.

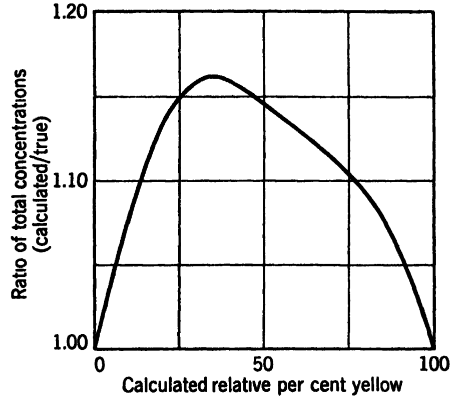

Fig. 7·44. Empirical correction curve for the two nonadditive components chrysophenine and diamine sky blue FF. (Total concentration 15 mg./l.)

Table 7·4 Determination of Empirical Corrections for Nonadditivity

Chrysophenine and diamine sky blue FF; total dye concentration 0.015 g./l.

Chrysophenine, %	*Diamine Sky Blue FF, %*	*Absorbancy Index*		*Calculated Concentrations*		*Calculated % Yellow*	*Calculated Total Dye Concentration, g./l.*
		430 mμ	650 mμ	*Yellow*	*Blue*		
100	0	0.560	0	100	0	100	0.0150
90	10	0.568	0.022	98	7	93	0.0158
80	20	0.518	0.043	92	16	86	0.0161
70	30	0.487	0.067	86	24	78	0.0165
60	40	0.448	0.092	78	33	70	0.0167
50	50	0.398	0.121	69	43	62	0.0169
40	60	0.347	0.151	60	54	52	0.0170
30	70	0.294	0.185	50	66	43	0.0173
20	80	0.223	0.224	36	80	31	0.0174
10	90	0.133	0.258	20	92	18	0.0167
0	100	0.025	0.280	0	100	0	0.0150

As an illustration of the use of the empirical correlation curves, consider the analysis of a mixture of chrysophenine and diamine sky blue FF having absorbancy coefficients at the two wavelengths of analysis such that the calculated concentrations are 0.0045 g./l. yellow and 0.0120 g./l. blue. This mixture of unknown concentrations thus calculates to be 36.7 relative per cent yellow, and 0.0165 g./l. total concentration of dye.

Reference to Fig. 7·44 shows that a mixture with an apparent relative per cent yellow of 36.7 will give a calculated total concentration too high by a factor of 1.16. The calculated total concentration, 0.0165 g./l., is divided by 1.16 to obtain 0.0142, which is the actual total concentration. Reference to Fig. 7·45 shows that a calculated relative per cent yellow of 36.7 corresponds to an actual relative per cent yellow of 25.0. The correct proportion in the mixture is thus 25.0 relative per cent yellow. Multiplying the true total by the true proportion gives 0.0036 g./l., the true concentration of yellow. The blue concentration is 0.0106 g./l. by difference from the true total concentration.

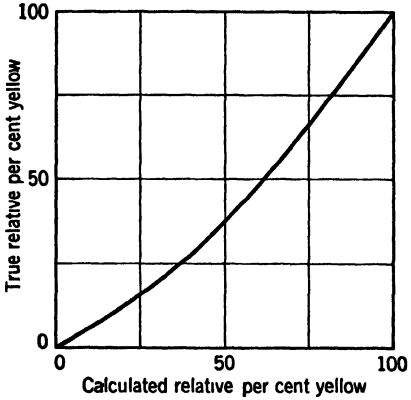

FIG. 7·45. Empirical correction curve for the two nonadditive components chrysophenine and diamine sky blue FF. (Total concentration 15 mg./l.)

Figures 7·44 and 7·45 apply only to a total true concentration of 15 mg./l. At a different concentration level, say 1.5 mg./l. total, different empirical correction curves may apply. Suitable corrections may be worked out by having a family of curves on Fig. 7·44 applying to different levels of true total concentration, and having a family of curves on Fig. 7·45 also applying to different levels of true total concentration. With such a family of curves, the true total concentration is first determined by successive approximations, any curve being used to get the first approximate total concentration; then a more nearly correct curve is used for the next approximation. The true relative proportion may then be determined directly from the correlation curve derived from mixes of the true total concentration.

In some commercial analyses most of the mixes will have about the same total concentration and relative proportion of components. For such special cases, in the event that absorbancy coefficients are not additive, an incremental method of determination of absorbancy indexes applicable to a particular mix is often the simplest method of analysis.

To derive the incremental equation, increments are added to equation (7·25), and then equation (7·25) is subtracted. Thus

$$(c_a + \Delta c_a)(a_s)_{a\lambda_1} + (c_d + \Delta c_d)(a_s)_{d\lambda_1} = (k_s)_{m\lambda_1} + \Delta(k_s)_{m\lambda_1}$$

$$c_a(a_s)_{a\lambda_1} + c_d(a_s)_{d\lambda_1} = (k_s)_{m\lambda_1}$$

Subtracting these two and repeating the process at wavelength λ_2 give equations (7·34).

$$\begin{aligned} \Delta c_a(a_s)_{a\lambda_1} + \Delta c_d(a_s)_{d\lambda_1} &= \Delta(k_s)_{m\lambda_1} \\ \Delta c_a(a_s)_{a\lambda_2} + \Delta c_d(a_s)_{d\lambda_2} &= \Delta(k_s)_{m\lambda_2} \end{aligned} \qquad (7\cdot34)$$

Solving these simultaneously gives equations (7·35), which are similar to equations (7·28).

$$\begin{aligned} \Delta c_a &= \Delta(k_s)_{m\lambda_1}\left[\frac{(a_s)_{d\lambda_2}}{|D|}\right] - \Delta(k_s)_{m\lambda_2}\left[\frac{(a_s)_{d\lambda_1}}{|D|}\right] \\ \Delta c_d &= \Delta(k_s)_{m\lambda_2}\left[\frac{(a_s)_{a\lambda_1}}{|D|}\right] - \Delta(k_s)_{m\lambda_1}\left[\frac{(a_s)_{a\lambda_2}}{|D|}\right] \end{aligned} \qquad (7\cdot35)$$

To apply the above equations, the absorbancy indexes are found with equations (7·34) by determining empirically the incremental change in the absorbancy coefficients $\Delta(k_s)_{m\lambda_1}$ and $\Delta(k_s)_{m\lambda_2}$ which results from a change in the concentration of each component (Δc_a and Δc_d). These values of the absorbancy indexes are then used to evaluate the coefficients of equations (7·35). The increments should be large enough to give reasonable accuracy in the determination of the absorbancy indexes but should be small enough to fall in a range where the absorbancy indexes are essentially constant.

When an unknown is measured, the differences in its absorbancy coefficients at the two wavelengths of analysis from the absorbancy coefficients of the standard known mixture are substituted in equations (7·35) and these, when solved, give the incremental concentrations. The incremental concentrations are added to the concentrations of the standard known mixture. A hypothetical numerical example is worked out in Table 7·5. The Δ values of Table 7·5 are obtained by subtract-

TABLE 7·5 NUMERICAL EXAMPLE OF INCREMENTAL ANALYSIS

Absorbancies neither linear with concentration nor additive.

Sample		*Absorbancy Coefficients*					
Ratio	Concentration	1	2	Δc_a	Δc_d	$\Delta(k_s)_{m\lambda_1}$	$\Delta(k_s)_{m\lambda_2}$
50% *A*, 50% *D*	0.0500	0.700	0.600	.	. .	. . . .	.. ...
47.6% *A*, 52.4% *D*	0.0525	0.695	0.650	0	0.0025	−0.005	+0.050
52.4% *A*, 47.6% *D*	0.0525	0.800	0.610	0.0025	0	+0.100	+0.010
Unknown		0.750	0.620			+0.050	+0.020

ing the various values from the standard mixture values given in the first line. The three known samples are necessary to set up the standard equations. Substituting the observed values in equations (7·34),

$$0 + 0.0025(a_s)_{d\lambda_1} = -0.005$$

$$0 + 0.0025(a_s)_{d\lambda_2} = +0.050$$

$$0.0025(a_s)_{a\lambda_1} + 0 = +0.100$$

$$0.0025(a_s)_{a\lambda_2} + 0 = +0.010$$

These give values for the absorbancy indexes as follows

$$(a_s)_{d\lambda_1} = -2.000$$

$$(a_s)_{d\lambda_2} = +20.00$$

$$(a_s)_{a\lambda_1} = +40.00$$

$$(a_s)_{a\lambda_2} = +4.000$$

These values, substituted in equations (7.35), give the standard equations for a ratio of 50 per cent A and 50 per cent D and a total concentration of 50 mg./l.

$$\begin{aligned} \Delta c_a &= 0.0252\Delta(k_s)_{m\lambda_1} + 0.0025\Delta(k_s)_{m\lambda_2} \\ \Delta c_d &= 0.0505\Delta(k_s)_{m\lambda_2} - 0.0050\Delta(k_s)_{m\lambda_1} \end{aligned} \qquad (7\cdot36)$$

Using equations (7·36), the unknown may be analyzed. Substitution gives the following:

$$\Delta c_a = (0.0252)(0.050) + (0.0025)(0.020) = 0.00131$$

$$\Delta c_d = (0.0505)(0.020) - (0.0050)(0.050) = 0.00076$$

The final results are then obtained by adding the increments to the standard concentration, and it is found that, for the unknown, $c_a = 0.0250 + 0.00131 = 0.0263$, and $c_d = 0.0250 + 0.00076 = 0.0258$.

It will be noted that the absorbancy coefficient at wavelength λ_1 of the second sample in Table 7·5 is less than that of the first, although it contains more total components. This is possible by a component interaction effect. It is this very effect, which may be expressed by saying that absorbancies are not additive, which limits the standard incremental equations (7·35) to mixtures in the approximate ratio and concentration of the standard curve.

The coefficients of equations (7·36) are not determined very accurately for two reasons: (1) because the incremental values used in calculating the absorbancy indexes are not known to many significant figures;

and (2) because equations (7·34) are not quite correct, since the absorbancy indexes are not constant even over the small range involved. If the incremental concentrations are increased, the accuracy of the incremental absorbancy coefficients will be increased, but equations (7·34) are not valid. If the increments of concentration are decreased so that equations (7·34) apply with practically no error, the accuracy of the incremental absorbancy coefficients is lost. A compromise value of incremental concentrations of about 10 per cent will often be found satisfactory. It should also be noted that the relatively inaccurate coefficients of equations (7·36) do not introduce much of an error in the calculated concentration because they are used to calculate only the incremental concentrations of the unknown.

7·46 Log A_s method, additive absorbancies. An analysis which corresponds to the data of Table 7·2 is illustrated by Figs. 7·46 and

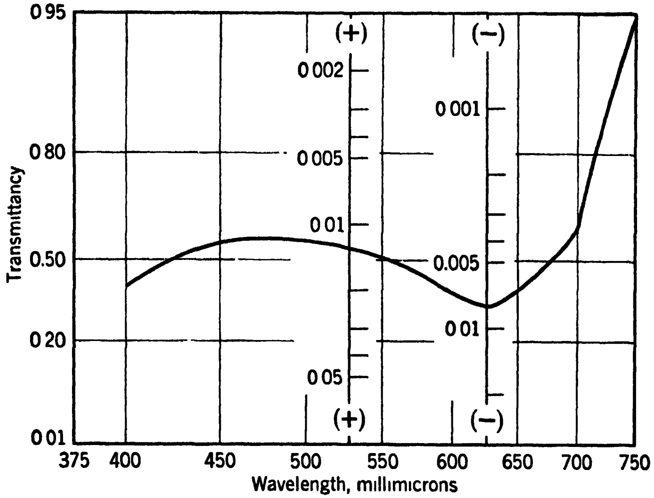

Fig. 7·46. Curve for log A_s method for two-component analysis of a mixture of diamine violet N and diamine green B. A solution of unknown concentration in 1.000-cm. cell; (+) scale placed so that 0.04912 falls at 0.10 transmittancy; (−) scale placed so that 0.01628 falls at 0.10 transmittancy; sum of scale intersections = concentration of violet.

7·47. The problem is to solve equations (7·29). The first term in solving for c_a in equations (7·29) is $0.04912(k_s)_{m\lambda_1}$. If the reference scale is placed vertically so that 0.04912 falls at 0.10 transmittancy, then the reading on the reference scale at the intersection of the plotted curve, measured in a 1.000-cm. cell, will be $0.04912(k_s)_{m\lambda_1}$. This is the position of the reference scale at 528 mμ in Fig. 7·46. Similarly, at 625 mμ the reference scale is placed so that 0.01628 falls at 0.10 transmittancy. Equations (7·29) direct that the one quantity is to be subtracted from the other, and accordingly the two scales are labeled + and − on the standard nomograph. If the curve of the unknown is traced on the standard nomograph, adjusted for cell length, Fig. 7·46 will be obtained. In this case the unknown is the mix of Table 7·2 adjusted vertically to the position it would plot if it had been measured

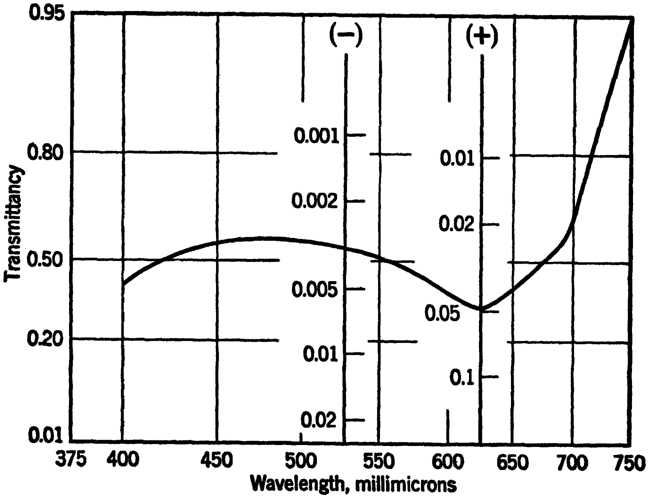

Fig. 7·47. Curve for log A_s method for two-component analysis of a mixture of diamine violet N and diamine green B. A solution of unknown concentration in a 1.000-cm. cell; (+) scale placed so that 0.10031 falls at 0.10 transmittancy; (−) scale placed so that 0.01246 falls at 0.10 transmittancy; sum of scale intersections = concentration of green.

in a 1.000-cm. cell. The intersection on the scale at 528 mμ marked + is 0.0137, and the intersection on the scale at 625 mμ marked − is 0.0080. The sum of these two, with proper regard for signs, is 0.0057 g./l.

In order to trace the curve at the vertical position it would occupy if it had been measured in a 1.000-cm. cell, the curve is traced so that its measured position intersects a reference scale at a reading corresponding to the cell length used and the adjusted position intersects the reference scale at a reading of 1. In practice the curves are not traced on the nomograph but are plotted on transparent paper and superimposed on the nomograph. Also, the scales need not be drawn on the nomograph; only one point must be indicated so that the reference scale may be placed properly. One nomograph with two scales is needed for component A. Another nomograph with two scales is needed for component D. The scales to solve for D are shown in Fig. 7·47.

Theoretically, the standard nomograph need consist only of four reference points to indicate the position at which to place the reference logarithmic scales. In practice, it is always well to perform a qualitative analysis first. This requires that the curves of the two components be drawn in full. A property of log A_s plots, if Beer's law is obeyed, and if the absorbancies are additive, is that, regardless of how two standard curves are adjusted vertically, the curve of a mixture of the two can be adjusted vertically so that it falls between the other two at every wavelength. For qualitative purposes the standard nomograph should show the two curves of the two components, and in general they should be arbitrarily adjusted so that they cross in as many points as possible. For instance, Fig. 7·48 shows the curves of the standards as they appear in Fig. 7·42. Curve M of Fig. 7·48 is the curve of a hypothetical sample submitted for analysis. When this curve M is placed at one intersection point by vertical adjustment, it falls above the other intersection point of the standard curves. If it is lowered to agree at the second intersection point, it falls below the first. Therefore, solution M fails to meet the test of falling everywhere between the two standard curves; it is not solely a mixture of A and D, and a spectrophotometric analysis will not give the right answer. Sub-

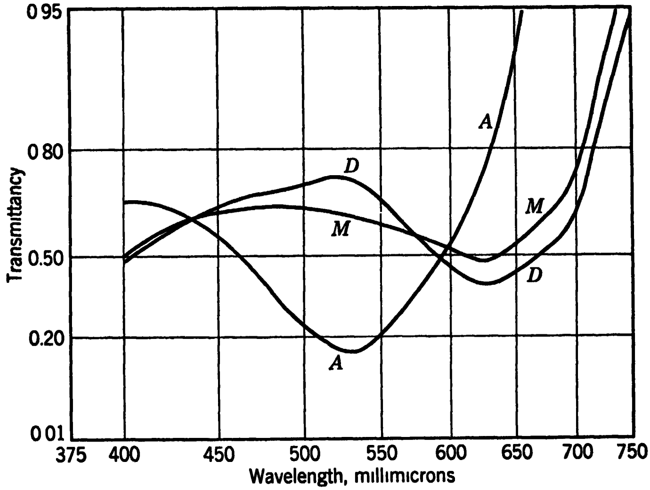

FIG. 7·48. Curves showing qualitative check for validity of two-component analysis. Since curve M cannot be made to fall between curves A and D at all wavelengths, it is not solely a mix of A and D, and an analysis in terms of these two will be in error.

stitution of observed absorbancies of M in equations (7·29) will give a numerical answer, but this calculated composition will be in error. It is possible to recognize this error algebraically by calculating the compositions at several wavelengths, in which case different compositions will be indicated by the absorbancies at the different wavelengths. However, the log A_s plots, with a little practice, will allow one to check at a glance approximately the qualitative composition and verify the validity of the two-component analysis about to be made. This qualitative check is one of the major advantages of the method.

7·47 Log A_s method, nonadditive absorbancies. In the event of nonadditivity of absorbancy coefficients, the log A_s method may still be used if calculations are made relative to an empirical mixture of similar composition. The absorbancy indexes applicable to deviations from a particular standard mixture are determined by the incremental method of evaluation described in Section 7·45. The reference marks are then placed on the standard curve in accordance with these values. To analyze a mixture of unknown composition the standard is subtracted from the unknown graphically at the two analysis wavelengths by the method described in Section 7·4, and the resulting two points are analyzed with the reference scales by the method described in Section 7·46. These calculated increments of concentration are added to the standard concentration. Such a method will give correct analyses only for mixtures close to the ratio and total concentration present in the standard curve mix.

7·48 Analyses at one wavelength. As interpreted thus far, the subscripts 1 and 2 in equations (7·28) have applied to one solution measured at two wavelengths. However, the mathematical relation still holds if the subscripts apply to two solutions measured at one wavelength if the two solutions are suitably related.

Equations (7·28) may be further simplified if in addition to having $(a_s)_{a\lambda_2} = 0$ it is also true that $(a_s)_{d\lambda} = (a_s)_{d\lambda_2}$. Then

$$c_d = \frac{1}{(a_s)_{d\lambda_2}} (k_s)_{m\lambda_2}$$

$$c_a = \frac{1}{(a_s)_{a\lambda_1}} [(k_s)_{m\lambda_1} - (k_s)_{m\lambda_2}] \qquad (7\cdot37)$$

One example of the use of equations (7·37) and only one wavelength is the method for ascorbic acid proposed by Johnson (*103*). In this analysis a is ascorbic acid; d is an absorbing nonoxidizable impurity. The first absorbancy coefficient of the mix is determined with the original sample; the second absorbancy coefficient, determined at the same wavelength, is determined after the sample has been oxidized. The ascorbic acid is oxidized to a nonabsorbing compound; hence $(a_s)_{a\lambda_2} = 0$. The nonoxidizable impurity is unchanged; hence $(a_s)_{d\lambda_1} = (a_s)_{d\lambda_2}$. Thus, equations (7·37) apply to the analysis.

In multicomponent analyses it is possible to measure some absorbancy coefficients at different wavelengths and some absorbancy coefficients with different solutions and combine the data to solve for concentrations.

As an example of a three-component analysis at two wavelengths and with two solutions the work of Holiday may be cited (*92*, *93*). Analyzing for tyrosine and tryptophan and unknown pigments, the investigator adjusts the *p*H of the solution to the acid side and measures it at 305 mμ. Under these conditions the tyrosine and tryptophan have no absorption, and the unknown pigments are evaluated. On the assumption that the absorption for the unknown pigments at both 280 and 305 mμ is not affected by changing the *p*H of the solution, the solution is made alkaline and measured at 280 and 305 mμ. The regular two-component equations are applied after the unknown pigment absorption has been subtracted.

As an example of a six-component analysis at three wavelengths with two solutions the work of Brice may be cited (*25*). Measurements are made at 232, 268, and 316 mμ on an initial solution of three fatty acids and again on a solution after treatment with alkali to induce conjugation of three additional components. This work is discussed in some detail in Section 7·59.

7·49 One component and impurity. Routine industrial analyses of organic material often consist of determining the concentration of batch after batch of material which varies slightly in its concentration and purity with regard to absorbing compounds. It is desirable to choose a wavelength for single-component analysis which is little influenced by the absorbing impurities and to report a quantitative measure of the absorbing impurities present. These two items are discussed below.

7·50 Choice of wavelengths. The determination of the optimum wavelengths to measure concentration of the main component least influenced by absorbing impurities, and the amount of impurity present least influenced by the main component, is accomplished in the same manner as discussed in Section 7·42, an additional difficulty being that ordinarily the absorption curve of the likely impurities is unknown and somewhat variable from batch to batch of main component. In general, more compounds absorb at shorter wavelengths than at long wavelengths. In the visual range a compound is more likely to have accidental impurities which are yellow or brown in color than to have blue impurities. Correspondingly, in the ultraviolet range the accidental impurities are more likely to absorb at 215 mμ than at 400 mμ. A likely curve shape for the impurity is therefore a line sloping from high transmittancy at long wavelength to low transmittancy at short wavelength. Such an impurity would shift the optimum wavelength for determination of concentration of main component slightly to the long-wavelength side of the absorption maximum.

The optimum wavelength for measurement of absorbing impurity is in a spectral region where the main component has a small absorbancy coefficient, and on the short wavelength side of that spectral region.

7·51 Impurity index, algebraic method. A quantitative measure of the amount of unknown absorbing impurities present may be designated an impurity index. In many industrial analyses of organic materials the nonabsorbing impurities, such as water and sodium chloride, are no detriment during the manufacturing process, but absorbing impurities indicate undesirable side reactions which may influence further processing. It is these that the impurity index measures.

A recommended impurity index, defined algebraically, is

$$(\text{I.I.}) = \frac{(k_s)_{m\lambda_1}}{(k_s)_{m\lambda_0}} - \frac{(a_s)_{a\lambda_1}}{(a_s)_{a\lambda_0}} \qquad (7\cdot38)$$

where (I.I.) = impurity index.

$(a_s)_{a\lambda_1}$ = absorbancy index of pure component a at wavelength λ_1.

$(k_s)_{m\lambda_1}$ = absorbancy coefficient of sample at wavelength λ_1.

$(a_s)_{a\lambda_0}$ = absorbancy index of pure component a at wavelength λ_0.

$(k_s)_{m\lambda_0}$ = absorbancy coefficient of sample at wavelength λ_0.

Wavelength λ_0 is that at which the concentration of a is determined. Wavelength λ_1 is that at which the impurity index is calculated.

7·52 Impurity index, log A_s method. If data are plotted on a log A_s basis, the method of drawing in a scale from which impurity index may be read is shown in Fig. 7·49. A horizontal line is drawn from the point on the standard curve where concentration is determined, 610 mμ in Fig. 7·49, to the wavelength chosen for the impurity index, 490 mμ in Fig. 7·49. A series of marks is made along the wavelength of the impurity index with the reference logarithmic scale, each mark being the sum of $(k_s)_{a\lambda_1}$ and (I.I.)$(k_s)_{a\lambda_0}$, where (I.I.) assumes various values. Each mark is labeled with its corresponding (I.I.) value.

To use the standard curve impurity index as drawn in Fig. 7·49, the curve of the sample plotted on transparent paper is placed above that of the standard curve and adjusted vertically until the sample curve is superimposed on the standard curve at 610 mμ. The intersection of the sample curve with the impurity index scale is then read off.

The greatest usefulness of the impurity index is to provide an arbitrary scale by means of which the relative purity of batches of organic material can be indicated numerically in routine industrial analyses. Equation (7·38) provides such a scale.

If it is assumed that the impurity arbitrarily evaluated by the impurity index has a horizontal curve of absorbancy coefficient, or merely that the absorbancy index of the impurity at wavelength λ_0 is equal to that at wavelength λ_1, which in turn is equal to the absorbancy index of the standard curve material at wavelength λ_0, or in other words that

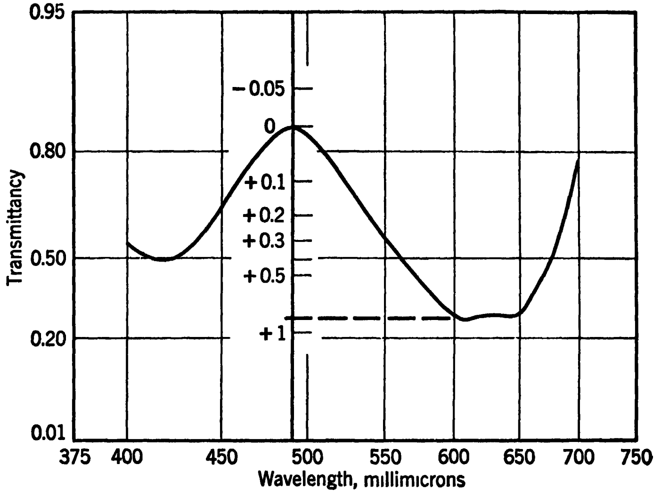

FIG. 7·49. Illustration of impurity index for alizarin cyanine green (C.I. 1078). The numbers at 490 mμ are the impurity index scale.

$$(a_s)_{u\lambda_0} = (a_s)_{u\lambda_1} = (a_s)_{a\lambda_0} \qquad (7\cdot39)$$

where u is the impurity, then the impurity index can be used to calculate the amount of the reported concentration of a in the sample which might be in error because of the impurities. This will be clarified by reference to Figs. 7·50, 7·51, 7·52, and 7·53, Table 7·6, and the following discussion.

Figure 7·50 represents a pure material a at a concentration of 1 g./l. Figure 7·51 shows a curve M which is the result of a mixture of 1 part of pure a and 0.1 part of the hypothetical impurity u. Figure 7·52 shows a curve M which is the result of mixing 1.0 part of pure a and 0.5 part of impurity u. In Fig. 7·53 curve M is a mix of 1.0 part a and 1.0

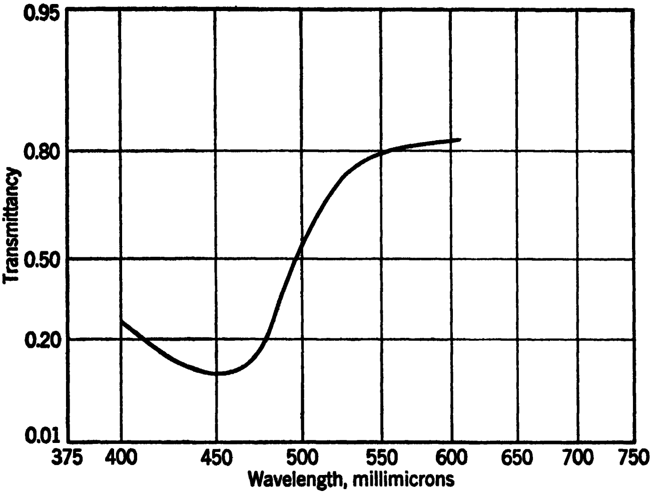

Fig. 7·50

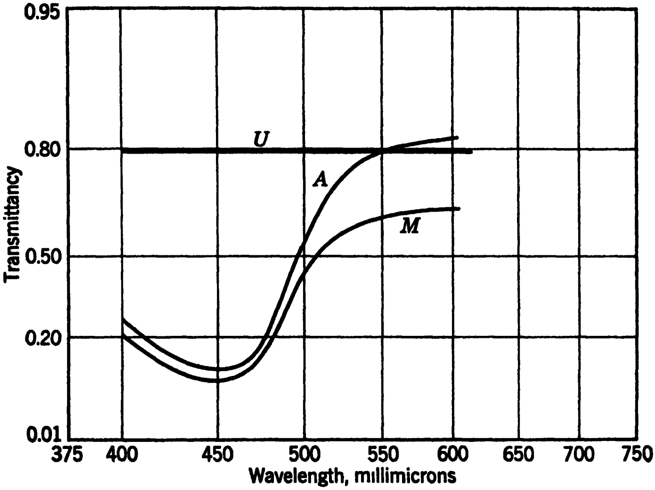

Fig. 7·51

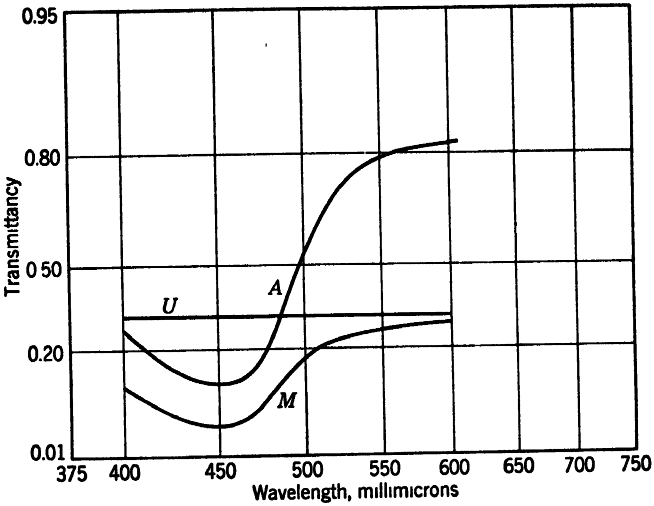

FIG. 7·52

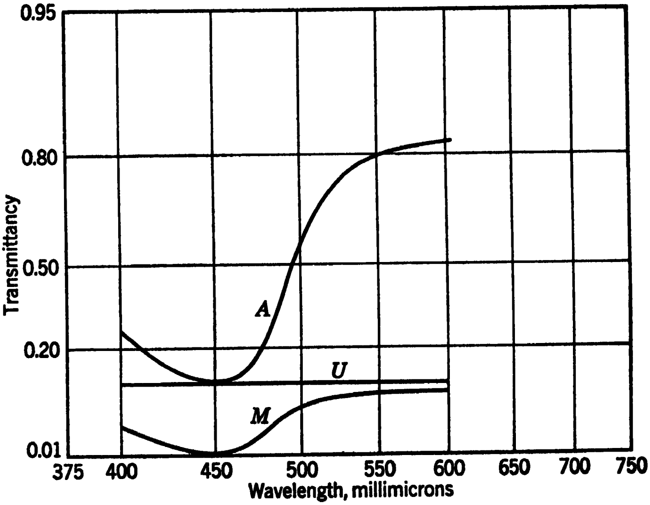

FIG. 7·53

FIGS. 7·50, 7·51, 7·52, and 7·53. Application of impurity index to correction in calculated concentration for absorption of "horizontal impurity."

A = absorption curves of pure component a;
U = impurity assumed present;
M = observed curves of mixes.

part u. The problem is to determine the amount of a present in each mixture, given the M absorption curves of Figs. 7·50 to 7·53. Columns 2 and 3 of Table 7·6 give the observed absorbancy coefficients of these four mixtures at the two wavelengths. If the ratio $(k_s)_{m\lambda_0}/(a_s)_{a\lambda_0}$ is calculated, which is the formula for calculating concentrations in a one-component system, the results listed in column 4 of Table 7·6 are obtained. They are greatly in error for large amounts of impurity such as the sample of Fig. 7·53. If the impurity index is determined, the values listed in column 5 will be obtained.

TABLE 7·6 IMPURITY INDEX CALCULATIONS

	k_s of Curve M				
Figure	λ_0	λ_1	*Apparent Concentration of M*	*Impurity Index at 600 mμ*	*Concentration of a in M **
7·50	1.0	0.1	1.00	0	1.00
7·51	1.1	0.2	1.10	0.082	1.00
7·52	1.5	0.6	1.50	0.300	1.00
7·53	2.0	1.1	2.00	0.450	1.00

* Corrected for horizontally absorbing impurity in accordance with equation (7·40).

For impurities having horizontal absorption curves, the impurity index is a measure of the amount by which the reported concentration will be in error. This possible error is calculated from the (I.I.) by means of equation (7·40). The correct amount of a present will be

$$c_a = \frac{(1 - \alpha)(k_s)_{m\lambda_0}}{(a_s)_{a\lambda_0}} \tag{7·40}$$

where

$$\alpha = \frac{\text{(I.I.)}}{[1 - (a_s)_{a\lambda_1}/(a_s)_{a\lambda_0}]} \tag{7·41}$$

Applying equation (7·40) to columns 4 and 5 gives column 6. It will be observed that in every case the calculation indicates correctly the amount of a present, regardless of the amount of the assumed impurity present.

The second application of the impurity index is thus to apply a correction to the apparent concentration calculated at wavelength λ_0 if it is assumed that the absorption of the impurity at wavelength λ_0 is the same as at wavelength λ_1. In some cases it might be decided to apply this correction in the routine interpretation of data and not bother to record the (I.I.) itself. This may be accomplished with some simplification. Equations (7·38), (7·40), and (7·41) may be combined and simplified to (7·42). [Note the resemblance to equation (7·23).]

$$c_a = \frac{(k_s)_{m\lambda_0} - (k_s)_{m\lambda_1}}{(a_s)_{a\lambda_0} - (a_s)_{a\lambda_1}} \tag{7·42}$$

In some cases $(a_s)_{a\lambda_1}/(a_s)_{a\lambda_0}$ may be so small that it may be assumed to be zero, and equation (7·41) reduces to α = (I.I.). Subject to this approximation, the (I.I.) becomes the fraction of the apparent concentration, $(k_s)_{m\lambda_0}/(a_s)_{a\lambda_0}$, which is caused by the impurity.

A third application of the impurity index is in quality-control programs, where mixing of lots is possible before further reaction. It may be found that a commercial process is best adapted to high yield if the material entered in the batch operation has a certain purity. This can be indicated by the concentration and the impurity index. Thus a process may be designed for best yields with material of 90 per cent, (I.I.) = 0.05. Two lots of material may be available, one analyzing 95 per cent, (I.I.) = 0.00, the other analyzing 85 per cent, (I.I.) = 0.10. If these lots are mixed in equal weights, the resulting mixture will analyze 90 per cent, (I.I.) = 0.05. The impurity index as defined above in equation (7·38) is an additive quantity, that is, the (I.I.) of a mix will change in direct proportion to the weights of the components. This means that mixes or blends can be made on the basis of (I.I.) in order to produce a mix of a desired (I.I.). This additive quality is a desirable feature of any impurity index and is possessed by (I.I.) as defined in equation (7.38).

7·53 Double impurity index. When the impurity index on one side of an absorption band is known, and it is desired to correct the concentration calculated at the absorption maximum for the unknown impurity present, it is only a fair guess that the impurity has the same absorbancy index at the wavelength of the absorption maximum as it has at the wavelength of the impurity index. In some cases it is possible to calculate the impurity index on both sides of the absorption band. It is then customary to assume that the impurity has an absorption curve which is linear when plotted on a graph with absorbancy as the ordinate and wavelength as the abscissa (*10, 25*). This is a much better guess.

In order to apply equation (7·40) to correct the apparent concentration for an impurity of this characteristic, it is necessary to use an α different from that of equation (7·41) in that it is calculated from both the $(\text{I.I.})_1$ obtained at the shorter wavelength, and the $(\text{I.I.})_2$ obtained at the longer wavelength. This α is calculated from the two (I.I.)'s with equation (7·43).

$$\alpha = \frac{W(\text{I.I.})_1(a_s)_{a\lambda_0} + (\text{I.I.})_2(a_s)_{a\lambda_0}}{W[(a_s)_{a\lambda_0} - (a_s)_{a\lambda_1}] + [(a_s)_{a\lambda_0} - (a_s)_{a\lambda_2}]} \tag{7·43}$$

where

$$W = \frac{\lambda_2 - \lambda_0}{\lambda_0 - \lambda_1}$$

To illustrate this, assume the data of Table 7·7. A pure compound *a* has absorbancy indexes at 260, 268, and 280 mμ of 140, 210, and 130, respectively. The impurity indexes are to be set up at 260 and 280 mμ while the concentration is to be determined at 268 mμ. The constants of equation (7·43) may be calculated from the data of the standard and are found to be

TABLE 7·7 ILLUSTRATION OF DOUBLE IMPURITY INDEX

	260 mμ	*268 mμ*	*280 mμ*
$(a_s)_a$	140	210	130
$(k_s)_m$	132	122	70
(I.I.)	0.413	. . .	−0.048

α = 0.65; concentration = 0.58 g./l.; concentration corrected = 0.20 g./l.

$$\alpha_{268} = 1.704(\text{I.I.})_{260} + 1.136(\text{I.I.})_{280} \tag{7·44}$$

An unknown sample *m* is found to have absorbancy coefficients of 132, 122, and 70 at wavelengths of 260, 268, and 280 mμ, respectively. On the basis of the absorption at 268 mμ, the apparent concentration of *a* in *m* is 122/210 = 0.58 g./l. This is believed to be in error because some impurities of unknown characteristics are believed to be introducing some absorption. The impurity index calculated by means of equation (7·38) is found to be 0.413 at 260 mμ and −0.048 at 280 mμ. The large impurity index observed at 260 mμ is evidence of foreign absorption. It is desired to assume that an impurity having a straight line absorption curve, when plotted on an absorbancy ordinate, is present and to calculate the amount of *a* present if this is the case. Accordingly, equation (7·44) applies, and α_{268} is found to be 0.65. The apparent concentration is 0.58 g./l.; but on correcting this in accordance with equation (7·40), it is found that the concentration of *a* present is 0.20 g./l.

To check this answer, 0.20 times the absorbancy indexes of *a* give absorbancy coefficients of 28, 42, and 26, respectively. Subtracting these from the observed absorbancy coefficients of *m* gives 104, 80, and 44, respectively. Plotting these latter values on an absorbancy-wavelength plot at 260, 268, and 280 mμ gives a straight line, the desired assumption for the characteristics of the absorption curve of the impurity.

It will be observed that there is quite a similarity between this method of double impurity index or correction for "linear background absorption on an absorbancy plot" and the absorbancy coefficient difference method discussed previously in Section 7·40, particularly the method wherein a component being analyzed was completely nonabsorbing for one measurement during which the absorbancy coefficient of the impurity was determined. The distinction between the two methods lies in the fact that in the absorbancy coefficient difference method the true absorption of the impurity is determined by direct measurement at the wavelength of concentration determination, whereas in the present double impurity index method the absorbancy coefficient of the impurity at the wavelength of analysis is inferred from data at the two neighboring wavelengths.

7·54 Multicomponent analyses. Multicomponent analyses involve an extension of the principles used in two-component analyses. As many absorbancy coefficient determinations are needed as there are components to be determined. These may be measured at different wavelengths or at the same wavelengths with related solutions, or combinations of both as discussed in Section 7·48. It is futile to speculate on the maximum number of components which can be determined. Special mixtures, of no practical interest, could be designed to provide analyses of a large number of components. In one practical analysis, six components have been determined with a high degree of accuracy (*25*).

7·55 Choice of wavelengths. In the conventional analysis wherein three components are to be determined from measurements at three wavelengths the first problem is to find three optimum wavelengths. These will vary somewhat, depending upon the composition of the mixture to be analyzed. A good practice is to assume a probable composition, one which is likely to occur in the majority of samples to be analyzed, and treat this as an entity. The wavelength of maximum ratio of absorbancy coefficient of component *a* to absorbancy coefficient of "probable mix" is one good wavelength. The similar maximum-ratio wavelengths for the other two components are two other good wavelengths. These wavelengths of maximum ratio may be determined by the methods described in Section 7·42. The three wavelengths so found should be modified in accordance with solution effects and the other factors discussed in Section 7·34.

In the case of four components the "probable mix" is determined for the four components, and the maximum absorbancy coefficient ratio of each component to the "probable mix" is found to arrive at the four wavelengths. Similar procedures apply to larger numbers of components.

7·56 Methods of analysis. If the absorbancies are additive, the methods of analysis consist merely in solving simultaneous equations, the number of equations corresponding to the number of unknowns. The various methods available for solving simultaneous equations were discussed in Section 7·44.

If the absorbancies are not additive, the most generally satisfactory method is the incremental method of analysis discussed in Section 7·45. This requires that a number of analyses be made on samples of fairly similar composition. Other methods may be used in special cases. For instance, the method of successive approximations, described for one component in Section 7·37, has been used for four components and analyses have been made within 30 minutes (*72*).

F·SPECIAL ANALYTICAL METHODS

7·57 Reaction rates. The idea of following the course of a reaction colorimetrically was proposed as early as 1903 (*118*). In some cases samples of the reaction mixture are removed at intervals of time and measured spectrophotometrically, the spectrophotometer being only an analytical instrument in the usual manner. In other cases the reaction is carried out in a solution cell in the spectrophotometer and the concentrations determined. Here, again, the spectrophotometer is only an

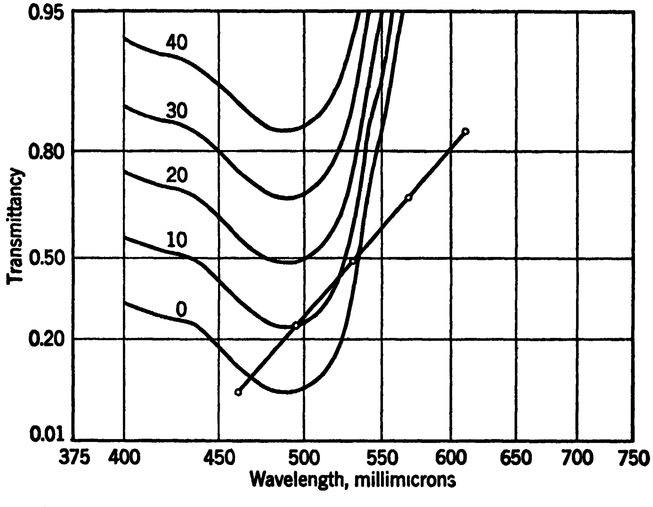

FIG. 7·54. Curves illustrating first-order reaction: hypothetical $a \rightarrow$ colorless d. Numbers indicate minutes of reaction time; points in circles are absorption maximum transmittancies plotted with time as abscissa.

analytical tool, but it is especially useful in this application because it determines composition quickly and without altering the sample. Examples of such applications are numerous (*44*, *64*, *87*, *106*, *144*, *179*). In analyzing these data it is customary to plot concentration versus time on special scales. For instance, in a first-order reaction, the plot of logarithm of concentration versus time will give a straight line the slope of which is proportional to the velocity constant.

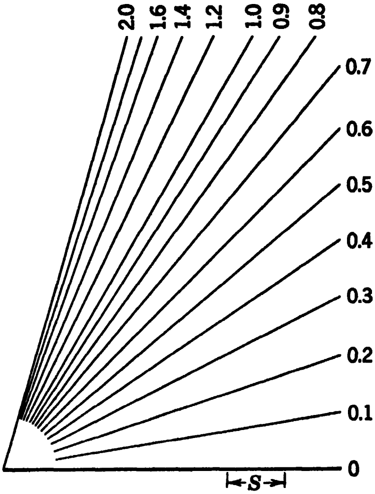

FIG. 7·55. Nomograph for determining velocity constant of first-order reactions.

$K = N/S$;
K = velocity constant (reciprocal seconds);
N = slope read from nomograph;
S = seconds plotted over unit of abscissa.

A nomographic method of determining velocity constants of first-order reactions from spectrophotometric data will be described here as an illustration of a special analysis. Figure 7·54 shows the five successive curves taken of a hypothetical reaction mixture at 10-minute intervals. This particular reaction is $a \longrightarrow d$, where the plotted curve at low transmittancies represents a, and d is a colorless product. The ordinate of the plot is logarithm of concentration at any one wavelength. If the reaction follows a first-order law, and curves are run at equal time intervals, it follows from a consideration of the first-order reaction law that an equal vertical displacement will result at equal intervals of time. The abscissa of the spectrophotometric plot may be labeled in units of time which should be evenly spaced. Points may then be made at the transmittancies as measured and the times when measured plotted on the abscissa. Such points are shown in Fig. 7·54. If a straight line may be drawn through these points, the reaction is a first-order reaction. The fact that the reaction is a first-order one may therefore be determined qualitatively directly from the transmittancy data, without converting them to concentrations.

The slope of the first-order reaction is related to the velocity constant and may be determined nomographically. Figure 7·55 shows a nomograph which has been prepared to calculate velocity constants. It consists of a number of lines of varying slope, each of which is labeled. To determine the constant of a first-order reaction such as that of Fig. 7·54, the transparent paper, on which the curves were plotted by the spectrophotometer, and on which the points of the wavelength of concentration determination were plotted displaced laterally according to time, is superimposed on the nomograph of Fig. 7·55 and displaced laterally until the time curve agrees with one of the sloping lines of Fig. 7·55. The number on the nomograph curve which agrees with the experimental curve is N in the formula $K = N/S$, where K is the velocity constant of the first-order reaction in units of reciprocal seconds, and S is the number of seconds in the unit abscissa interval. In this way the quantitative evaluation of a first-order reaction may be made without so much as calculating concentrations.

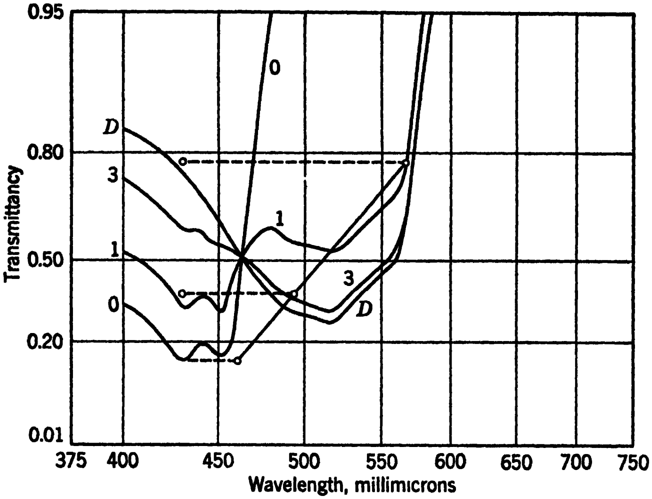

FIG. 7·56. Curves for hypothetical first-order reaction: $a \rightarrow d$.

0 = reaction mixture at zero time, pure a;
1 = reaction mixture after 10 minutes;
3 = reaction mixture after 30 minutes;
D = pure d.

Points at 430 mμ are absorption of a calculated by correcting curves 1 and 3 for amounts of d present; points on straight line are 430-mμ points plotted on an abscissa linear in time.

Automatic spectrophotometers have been made (*186*) which turn the recording drum linearly with time, recording at one wavelength. In cases such as Fig. 7·54, this gives a plotted curve from which the qualitative identification and quantitative evaluation of a first-order reaction may be carried out graphically in a matter of a few seconds per analysis.

Extension of this principle to second-order reactions with log time abscissa is obvious.

A time drive to plot changes in transmittancy with time at one wavelength fails to yield much useful information in cases other than the simple one of formation or disappearance of a colored material. Where one colored compound is formed at the expense of another, the simple time drive does not bring this out. If the complete spectrophotometric curves are run at intervals of time, a combination of the graphical addition or subtraction of curves, as discussed in Section 7·4, and the lateral displacement of points on a time abscissa may be useful. This is illustrated by the data of Fig. 7·56. This is a hypothetical case in which compound *a* is reacting to yield compound *d*. The curve of pure *a* is known and corresponds to curve 0 of Fig. 7·56. The curve of pure *d* is also known and corresponds to curve *D* of Fig. 7·56. Curves 0, 1, and 3 were run at three time intervals during the reaction, curve 0 being the start of the reaction, curve 1 after 10 minutes, and curve 3 after 30 minutes. Analyses for compound *a* are made at 430 mμ, but compound *d* also absorbs here, so that the observed absorbancy coefficients must be analyzed in accordance with a two-component system. Since compound *a* does not absorb at 520 mμ, the amount of *d* present may be determined at 520 mμ and the observed absorbancy coefficient at 430 mμ corrected graphically for absorption of *d*. These corrected points are plotted on the 430-mμ ordinate. Of course, curve 0 needs no correction for *d* since none has been formed at the start. The corrected points at 430 mμ are displaced laterally and plotted on a time abscissa. These points are also shown in Fig. 7·56. A line drawn through these points is a straight line, indicating a first-order reaction, and, by comparing the slope with Fig. 7·55, the reaction constant can be determined, the whole operation being nomographic.

7·58 Optical rotation. Spectrophotometers that operate on the principle of polarization of light, such as those using a Martens photometer, may, as has been pointed out by Shurcliff (*177*) and Brode (*36*), be used to measure rotatory dispersion curves. Such data are not spectrophotometric absorption data and therefore will not be treated. It may be mentioned that some compounds have characteristic curves; for instance, ethyl pyroglutamate reverses its direction of rotation and at one wavelength in the visual is nonrotatory. Also, if these data are plotted on a log of optical rotation basis, the curve shape obtained is independent of concentration and may be used for qualitative identification.

G·SPECIFIC APPLICATIONS

The previous sections of this chapter have presented the methods of analysis which are used to obtain useful information from spectrophotometric data. The present section is intended to review briefly some of the fields in which spectrophotometry has proved useful. A number of books and review articles in the literature discuss applications (*7*, *31*, *33*, *42*, *107*, *134*, *135*, *136*, *140*, *153*, *196*).

7·59 Fats and oils. Saturated hydrocarbons, alcohols, ethers, and amines are so transparent at wavelengths longer than 210 mμ that any definite absorption exhibited by these compounds is an indication of impurities. Unsaturated compounds of these types, if unconjugated, that is, if the ethylene linkage is separated from any other ethylene or carboxyl group by a —CH_2— group, are essentially nonabsorbing also. The absorption maximum of the isolated ethylene group is apparently close to 210 mμ, because in compounds containing a great many ethylene groups there is some evidence of absorption at this wavelength. Conjugation of double bonds produces absorption bands at longer wavelengths, two conjugated bonds giving an absorption maximum at approximately 230 mμ, three bonds at approximately 270 mμ, and four bonds at approximately 310 mμ. This is illustrated by Fig. 7·57. Many fats and oils are compounds of this type.

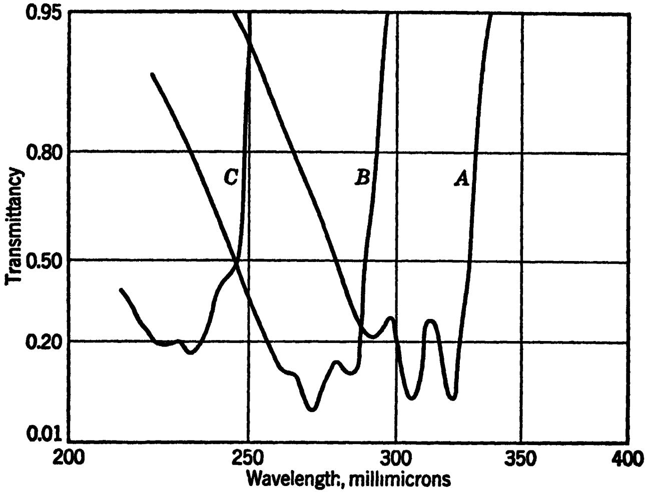

FIG. 7·57. Absorption spectra of some pure fatty acids.

A = parinaric acid (4 conjugated double bonds);
B = alpha-eleostearic acid (3 conjugated double bonds);
C = $\Delta^{10,12}$-linoleic acid (2 conjugated double bonds).

Solvent = ethanol; k_s = 189 at 270 mμ for *B*; see Kass (*107*).

The exact wavelength of the maximum absorption and the value of the absorbancy coefficient at that point are affected by the structure of the remainder of the molecule, but in the absence of other characteristically absorbing groups the spectrophotometric curves of unrelated compounds are remarkably alike. This is illustrated by Fig. 7·58, which shows the curves of dimethylbutadiene, $\Delta^{9,11}$-linoleic acid, and $\Delta^{10,12}$-linoleyl alcohol. The curves of Fig. 7·58 are not exactly alike, but the absorption in each case is attributed to the conjugated diene group, which alone is common to all three molecular structures, and it may be said in general that the absorption resulting from this type of grouping has its maximum at about 230 mμ. The qualitative analytical possibilities of this are well illustrated by the work of Kass and coworkers (*108*). Linoleyl alcohol had previously been assigned the incorrect structure of 9,12-octadecadien-1-ol. The absorption spectrum (curve *C*, Fig. 7·58) showed the presence of a dienoic conjugation. This explained some other properties of the alcohol which also were inconsistent with the structure first proposed.

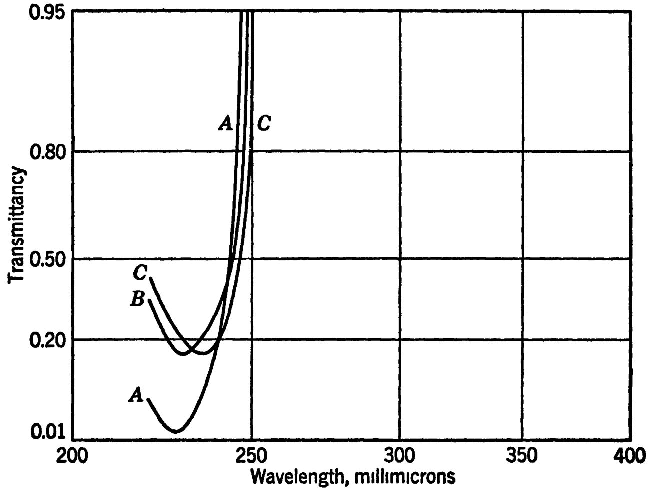

FIG. 7·58. Absorption spectra of some conjugated dienoic compounds.

A = dimethylbutadiene;
B = $\Delta^{9,11}$-linoleic acid;
C = $\Delta^{10,12}$-linoleyl alcohol.

Solvent = ethanol; k_s = 302 at 229 mμ for *A*; see Kass (*107*).

The fact that triple conjugation results in a characteristic absorption at about 270 mμ was utilized analytically by Steger and van Loon (*187*), who showed that alpha- and beta-licanic acids did not have a previously assigned structure but must possess three conjugated double bonds. The characteristic absorption of quadruple conjugation at about 310 mμ was used by Kaufmann and coworkers (*109*) to infer the structure of parinaric acid by its similarity to the known compound decatetraene.

The analytical possibilities of conjugation have been utilized in biochemical work. In metabolism the rat attacks only one of the bonds in eleostearic acid at a time, leaving the other two for a sufficient period to be measured spectrophotometrically (*141*).

It must be understood that spectrophotometry is only a tool to assist in these structural determinations and is supplementary to other chemical tests. An observed absorption of the type just discussed gives no indication of molecular weight or chain length, since in an homologous series the same absorption band is observed. The method is also limited by the fact that substitution of certain groups on the carbon atoms of the conjugated system will change the absorption characteristics (*18*). The geometrical configuration of the unsaturated system will also affect the exact location of the absorption maximum, but these differences are not large enough to invalidate the identification.

The quantitative analytical possibilities may be illustrated by the complex analysis of animal fats and soaps described by Brice and coworkers (*25*, *26*), who used the absorption possessed naturally by acids to determine three components and then developed further absorption by isomerization of unconjugated, unsaturated acids into their conjugated, absorbing form, which they measured to arrive at concentrations of three additional components, thus carrying out a six-component analysis in steps.

The first compound to be determined is conjugated tetraene acids. The mixed fatty acids are dissolved in alcohol or neohexane, and the absorbancy coefficient determined at 316 mμ. A comparison of the curves of many practical samples of interest with the standard curve of alkali-isomerized arachidonic acid usually shows that the tetraene acid absorption is obscured by other absorbing bodies which are believed to consist of oxidation and polymerization products, pigments, and other unknown compounds. Accordingly, the absorbancy coefficient is too large. It is corrected for "linear background absorption" (see Section 7·53). Impurities are estimated from data at 310 and 322 mμ. The corrected absorbancy coefficient divided by the absorbancy index of the standard gives the first result.

The second determination is percentage of conjugated triene acids. The absorbancy coefficient is determined at 268 mμ and corrected for impurities evaluated at 262 and 274 mμ. Although the conjugated tetraene compounds theoretically interfere and make this a two-component analysis, actually the interfering effect is small and the amounts of tetraene acids encountered are small, so that this coefficient is directly divided by the absorbancy index of the standard beta-eleostearic acid.

The third determination is percentage of conjugated diene acids. The absorbancy coefficient is measured at 232 mμ and corrected by a small amount, empirically determined, to allow for absorption of compounds such as methyl stearate. Although both the conjugated tetraene and conjugated triene acids interfere and make this a theoretical three-component system, the interference is slight and the amounts present are small, so that the conjugated diene acids are determined by dividing directly the observed absorbancy coefficient by the absorbancy index of the standard of $\Delta^{10,12}$-linoleic acid.

The fourth determination is arachidonic acid. All the nonconjugated unsaturated fatty acid constituents are converted to their conjugated isomeric form by an alkali treatment in glycerol. These acids now absorb in the ultraviolet. The absorbancy coefficient is measured at 316 mμ and corrected for impurities determined at 310 and 322 mμ. The coefficient previously observed for the naturally occurring tetraene acids is subtracted, and the remainder is proportional to the arachidonic acid present and is determined by reference to the curve of the alkali-isomerized arachidonic acid.

The fifth determination is nonconjugated linolenic acid. The absorbancy coefficient of the alkali-isomerized sample is measured at 268 mμ and corrected for impurities estimated from 262 and 274 mμ. It is reduced by subtracting the coefficient contributed by the naturally occurring triene acids. This coefficient, together with the coefficient of the alkali-conjugated arachidonic acid, is substituted in a two-component system equation.

The sixth determination is linoleic acid. The absorbancy coefficient is measured at 232 mμ, and the absorbancy coefficient of the sample before alkali isomerization subtracted. This remaining part is then substituted in the equation relating concentration of linoleic acid to absorbancy coefficients at 232, 268, and 316 mμ, along with the absorbancy coefficients of the triene and tetraene acids.

As an additional example of quantitative analysis, a complete analysis for saturated, oleic, linoleic, and linolenic acids may be carried out by a combination of chemical tests and measurement of absorption developed by isomerization (*23*, *147*). When linoleic acid is isomerized by treatment with potassium hydroxide in solution in ethylene glycol at 180°C. and subsequently suitably diluted with ethanol for spectrophotometric measurement, a strong absorption band develops at 230 mμ, which is characteristic of diene conjugation. When linolenic acid is similarly treated, the characteristic absorption at 270 mμ of a triene conjugation develops. Saturated acids and oleic acid develop no absorption by this treatment.

The analysis of an unknown vegetable oil or fatty acid mixture thus consists of subjecting the mixture to the isomerizing process and analyzing the two-component system spectrophotometrically for amounts of linoleic and linolenic acids present. The iodine number of the original acids is then determined titrimetrically, and the oleic acid determined by difference to make up the found iodine number. The saturated acids are then obtained by difference to make up the original weight of sample. A complete analysis requires about 0.2 g. of vegetable oil, and the average percentage error reported is ±1.33 for linolenic acid and ±1.83 for linoleic acid. The method has been extended to include animal fats by determining the interference effects of arachidonic acid on the specific absorption coefficients of the other acids (*11*).

The application of ultraviolet spectrophotometry to the determination of conjugation in drying oils, which is of importance in the paint industry, has been described by Kass (*107*). Spectrophotometric "dienometry" may detect as little as 0.1 per cent conjugation in mixtures with nonabsorbing materials. The exact identification of the conjugated compounds is not always possible or indeed necessary to get a useful result. For instance, dehydrated castor oil may be analyzed for the concentration of acids with two conjugated double bonds. It is customary to express the analytical result in terms of concentration of $\Delta^{9,11}$-linoleic acid because this acid has been prepared in pure form and its spectral curve determined. Dehydrated castor oil contains two geometrically isomeric $\Delta^{9,11}$-linoleic acids. Neither one of them is the same as the above product, which is made by the dehydration of ricinelaidic acid, and neither one has been purified so that a standard spectrophotometric curve could be run. Hence, the analysis of dehydrated castor oil on the basis of the $\Delta^{9,11}$-linoleic acid is only an approximation. Nevertheless it is a good enough approximation for practical purposes because the isomeric acids have fairly similar absorption curves.

Figure 7·59 shows the curves which are necessary for an analysis of dehydrated castor oil to determine di- and triconjugated acids present. Curve *A* is the standard curve of $\Delta^{9,11}$-linoleic acid with a characteristic absorption maximum at 232 mμ, where its absorbancy index is 115. Curve *B* is the standard curve of alpha-eleostearic acid with a characteristic absorption at 270 mμ, where its absorbancy index is 189. The absorbancy index of curve *A* at 270 mμ is practically zero. Curve *B* has an absorbancy index of 12 at 232 mμ. This may not be neglected but must be taken into consideration for accurate results. Equations (7·31) apply to this two-component analysis. Curve *C* is the curve of a particular sample of dehydrated castor oil which analyzed 0.1 per cent conjugated trienoic acids and 22 per cent diene conjugation.

As further examples of the value of the method, the spectrophotometric curves have also been used to determine the purity of acids prepared by different methods. For instance, it has been shown that the recrystallization from corn oil of linoleic acid gives a product that is more nearly free of conjugation than does the debromination of the tetrabromide (*37*).

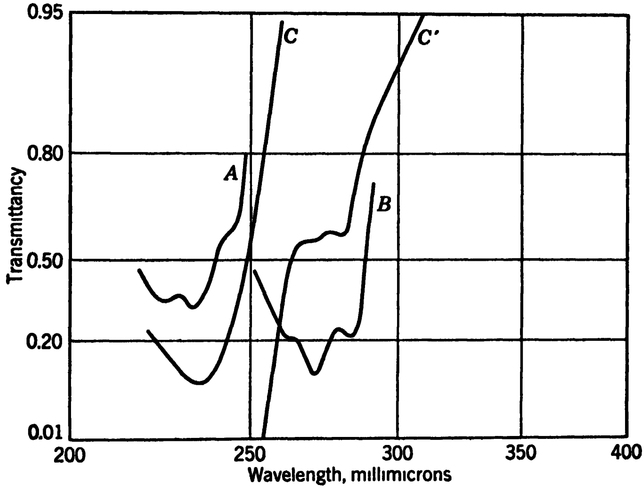

FIG. 7·59. Curves for analysis of dehydrated castor oil.

A = $\Delta^{9,11}$-linoleic acid (5 mg./l.);
B = alpha-eleostearic acid (5 mg./l.);
C = dehydrated castor oil (50 mg./l.);
C' = dehydrated castor oil (1000 mg./l.).

Solvent = ethanol; see Kass (*107*).

The change in the absorption curve of fatty esters has been related to oxygen absorption (*94*). The auto-oxidation of beta-eleostearic acid has been followed (*24*).

7·60 Vitamins, hormones, and enzymes. Spectrophotometry has played an outstanding role in the chemistry of vitamins, hormones, and enzymes. Qualitatively the absorption spectra have been of great assistance in helping to determine the molecular structure, and quantitatively they have been used to determine the amounts present in numerous academic and industrial problems. Good reviews of the subject have been prepared by Brode (*33*), Morton (*154*), and Miller (*140*), and a few examples have been chosen from this field.

Vitamin A is a polyene alcohol synthesized by animal organisms from a number of provitamins, all of which are carotenoids. Spectrophotometric data have been of assistance in characterizing carotenoids. For instance, three of the isomeric carotenes, which are designated alpha, beta, and gamma, and which collectively are an important pigment in green vegetation, have absorption maxima in carbon disulfide as follows: alpha, 511, 478, 446 mμ; beta, 520, 485, 451 mμ; and gamma, 533, 496, and 463 mμ (*154*). In the historical development of vitamin A studies it was puzzling that food supplements of vegetable products prevented the symptoms of deficiency from appearing while the "fish-oil vitamin A" was not detectable in plant products. Moore (*150*) was influential in solving this dilemma by showing, by means of ultraviolet absorption spectra of extracts of livers of experimental animals, that addition of carotene to vitamin A-free diets brought about an increase of vitamin A in the liver.

The interaction of fish-liver oils and antimony trichloride produces a blue color with an absorption maximum at 620 mμ, characteristic of vitamin A. In addition, some fish-liver oils, notably from fish caught in Russian rivers, show an absorption maximum at 693 mμ, which has been ascribed to a closely related compound which has been called vitamin A_2. Thus, spectrophotometry has assisted in the qualitative determination of the complexity of vitamin A. Determinations of the relative amounts of vitamin A and vitamin A_2 have been carried out by two-component analyses (*119*).

One analytical problem is the determination of vitamin A in such materials as fish-liver oil (*214*). This may be complicated by oxidation (*82*). Two spectrophotometric methods are available. One is to measure the ultraviolet absorption directly, and the other is to develop a blue color with antimony trichloride (*160*). Figure 7·60, curve *A*, shows the absorption curve of cod-liver oil. The curve is the resultant of the actual absorption of vitamin A and its esters, and irrelevant absorption due to miscellaneous inactive substances. A direct measurement at the absorption maximum at 325 mμ is only an indication of the upper limit of vitamin A present. It is therefore necessary to extract carefully the nonsaponifiable fraction, whereby the irrelevant absorption is removed. Curve *B* of Fig. 7·60 shows the same cod-liver oil as curve *A*, but only the nonsaponifiable fraction has been measured. The generally accepted datum assigns to pure vitamin A an absorbancy index of 182.5 at 324 mμ in diethyl ether, thus enabling one to determine the concentration by direct ultraviolet absorption of the purified product.

A second method of analysis for vitamin A is to develop the blue color with antimony trichloride in chloroform solution (*43*). This shows an absorption band at 617 mμ, which must be measured within 15 seconds of mixing, as the color is transient. When carried out on the natural oils, the color test is seriously affected by the presence of natural inhibitors (*152*). Thus the result obtained is too low. Saponification removes the inhibitors of the color test, and results obtained on the nonsaponifiable fractions of fish-liver oils by the color test and by direct ultraviolet measurement are in agreement. The generally accepted absorbancy index is 600 at 617 mμ for the color developed by reaction of antimony trichloride with "pure" vitamin A.

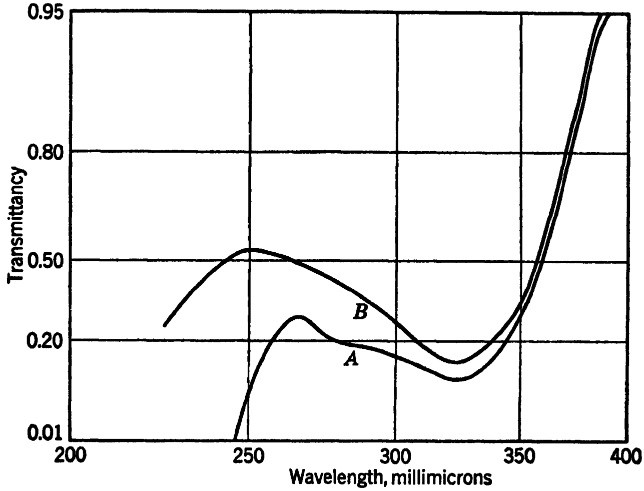

FIG. 7·60. Curves for vitamin A in cod-liver oil.

A = cod-liver oil;

B = nonsaponifiable fraction of cod-liver oil.

Solvent = ethanol; a_s = 0.16 for *B* at 325 mμ; see Morton (*154*).

A second analytical problem is the determination of carotene in dried grass and similar products. Freshly cut and artificially dried grass retains much carotene, and this is important because if cattle and chickens are fed such food in the winter the provitamin and vitamin A content of milk and eggs will approach that attained in summer when green foodstuffs are available. This in turn provides provitamins and vitamin A for human beings. Fortunately for simplicity in the analysis, the provitamin of grasses is almost entirely beta-carotene. Since the various carotenes possess different potencies as provitamins, the determination of potency in plants with substantial amounts of several carotenes depends upon a multicomponent spectrophotometric analysis followed by multiplication of each component by a potency factor.

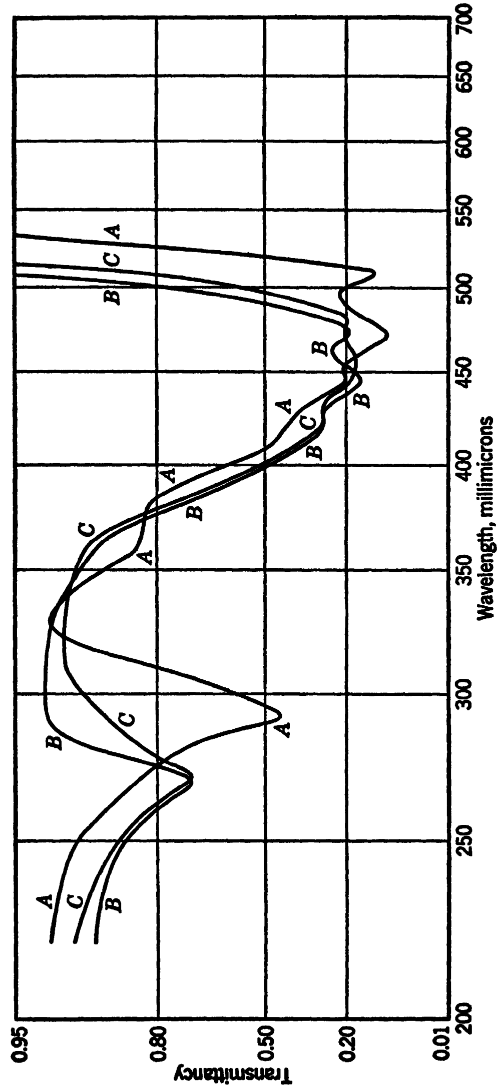

Fig. 7·61. Absorption spectra of carotenoids.

A = lycopene;
B = alpha-carotene;
C = beta-carotene.

Solvent = 80 per cent ethanol, 20 per cent diethyl ether; see Miller (*138*).

The problem consists in extracting the pigment, separating it from interfering pigments such as the "xanthophylls," and determining the carotene colorimetrically (*84*). The absorption curve of beta-carotene is given in Fig. 7·61.

A third analytical problem is the determination of both carotene and vitamin A in products such as butter where they occur together

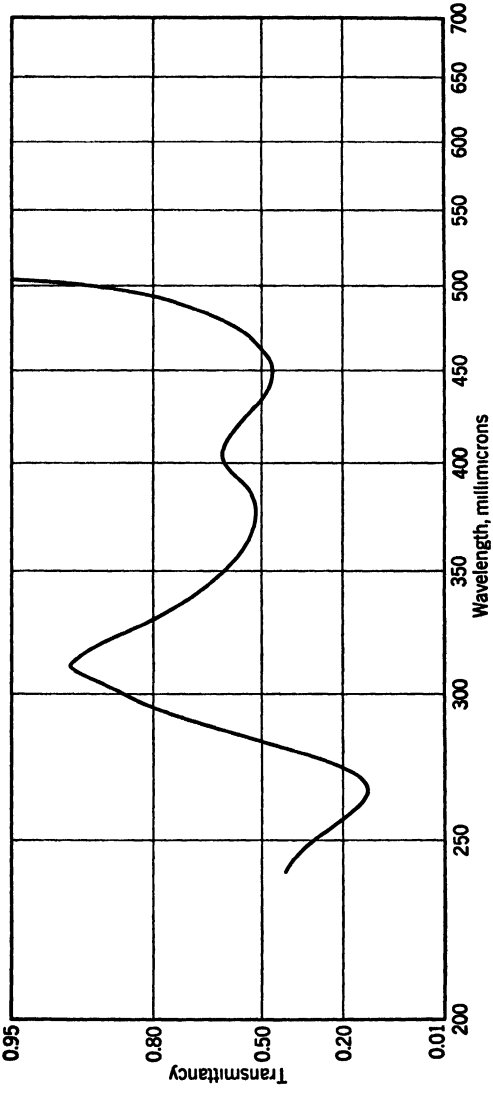

Fig. 7·62. Absorption spectrum of riboflavin.

a_s = 178 at 265 mμ; see Warburg and Christian (*201*).

(*215, 216*). Two methods of analysis are available. One is the direct spectrophotometric measurement and involves extracting the nonsaponifiable fraction of butter with diethyl ether and measurement at 324 mμ to estimate vitamin A. This value is usually somewhat high because of absorption due to carotenoids and other contaminants of unknown nature. Azo dyes which are used to color butter (F.D. and C. Yellow Nos. 3 and 4) also interfere somewhat. The ether solution may be measured for total carotenoids at 437 mμ, since at this wavelength the absorption spectra curves of pure beta-carotene and pure neobeta-carotene cross. The absorbancy index of each is 204 at 437 mμ. The total carotenoid result is in error because of absorption by carotenols and other pigments which vary, depending upon the food of the cow. To arrive at an analysis of carotene, the carotenols (and azo dyes) may be removed by a diacetone alcohol extraction.

In the indirect method the carotene is estimated the same way, but the vitamin A is treated with the antimony trichloride reagent to develop the blue color, which is measured at 620 mμ. Since the azo dyes do not interfere, this is the preferred method for commercial butters.

7·61 Vitamin B. Thiamine is the antiberiberi component of the vitamin B complex. The determination of the structure of thiamine has been discussed in Section 7·31 as an outstanding example of the usefulness of spectrophotometry in qualitative analysis.

Although thiamine possesses a characteristic absorption spectrum, it is usually estimated by a fluorimetric method, since in alkaline solution potassium ferricyanide oxidizes thiamine to thiochrome, a pale yellow compound characterized by an intense blue fluorescence (*51*, *161*). The color developed by coupling thiamine with a diazotized aromatic amine, such as sulfanilic acid, has also been suggested as a method (*112*).

Riboflavin or vitamin B_2 is a component of the oxidation-reduction system of cells. It has the absorption spectrum shown in Fig. 7·62, and this was of assistance in establishing its structure (*201*). For spectrophotometric quantitative analysis it has been found more convenient to convert the compound to lumiflavin by irradiation in alkaline solution and to measure the lumiflavin at 470 mμ (*115*).

Pyridoxine or vitamin B_6, an antidermatitis factor, has a characteristic absorption curve which was of assistance in determining its structure (*188*). Color tests have been proposed for its estimation (*172*, *192*).

7·62 Vitamin C. Ascorbic acid, the antiscorbutic vitamin, has the absorption spectrum shown by curve *A* of Fig. 7·12. The acid is easily oxidized to give a product having no appreciable absorption at wavelengths longer than 200 mμ. This property, loss of absorption by oxidation, has been made the basis of the quantitative determination of ascorbic acid in eye fluids which also contain other absorbing compounds (*103*).

One portion of the eye-fluid sample is measured directly, being careful to avoid oxidation. Another portion of sample is oxidized, catalyzed by copper (II) ion, and measured. The absorption shown by the second solution is that contributed by the components of the eye fluid other than ascorbic acid. Subtracting this absorbancy coefficient from the absorbancy coefficient of the first sample gives the absorbancy coefficient proportional to the ascorbic acid present.

Ascorbic acid may also be estimated by color development reactions. It quantitatively reduces ferribipyridine sulfate to the red ferrobipyridine ion, which may be evaluated at 510 mμ (*113*). It also quantitatively reduces 2,6-dichlorophenol indophenol to a colorless product, and the loss in color may be measured at 520 mμ (*86*). This latter determination is especially interesting, as it illustrates the application of reaction rates to quantitative analysis. In the analysis of blood plasma for ascorbic acid there may be present other materials, such as cysteine, which are also capable of reducing the dye 2,6-dichlorophenol indophenol (*145*). However, since the reaction with ascorbic acid is very rapid in acidic solution, being completed in less than 30 seconds, and the reaction with cysteine is slow, the analysis may be carried out.

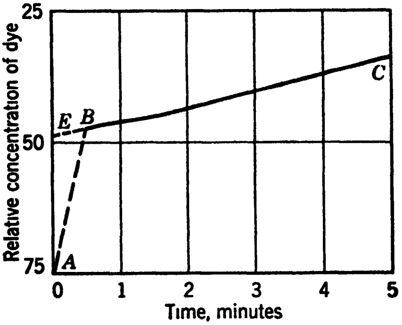

FIG. 7·63. Graph for the determination of ascorbic acid by reduction of the dye, 2,6-dichlorophenol indophenol. $A - B$ = reduction by ascorbic acid; $B - C$ = reduction by cysteine. See Mindlin and Butler (*145*).

A solution of the dye is prepared, and its absorbancy coefficient measured. An equivalent solution, except for the addition of the impure ascorbic acid, is prepared and measured at half-minute intervals of time. Figure 7·63 illustrates the data obtained when the impure ascorbic acid sample consisted of 0.01 mg. of ascorbic acid and 0.20 mg. of cysteine hydrochloride. The portion of the curve from A to B of Fig. 7·63 represents primarily the loss of dye concentration resulting from the oxidation of the ascorbic acid. This reaction is complete by B, and the portion of the curve from B to C therefore represents loss in dye concentration brought about by oxidation of cysteine. The curve BC may be extrapolated to zero time. When the data are plotted on a logarithm of concentration ordinate and time abscissa, first-order reactions will plot as straight lines and extrapolation may be facilitated. The ascorbic acid concentration may be determined as

$$c_a = c_d S(A - E)$$

where c_a = concentration of ascorbic acid.
c_d = concentration of dye initially.
S = stoichiometric conversion factor.
$(A - E)$ = loss in concentration.

Alternatively, the reduction of dye by other compounds can be stopped by extracting it with xylene after the ascorbic acid has had 15 seconds to react (*157*).

7·63 Vitamin D. Vitamin D is a complex vitamin, components of it being produced by ultraviolet irradiation of provitamins. Ergosterol is provitamin D_2, which, upon irradiation, yields calciferol, which is vitamin D_2. 7-Dehydrocholesterol is provitamin D_3, which, upon irradiation, yields vitamin D_3. Vitamin D_3 is similar to vitamin D_2 except that a double bond present in D_2 is saturated with hydrogen in D_3. Interference with the quantitative analysis is encountered both in the direct ultraviolet absorption measurement and in the antimony trichloride color development (which gives an absorption maximum at 500 mμ with vitamin D) by presence of vitamin A. It is essential to separate vitamin A from vitamin D by an adsorption before the analysis.

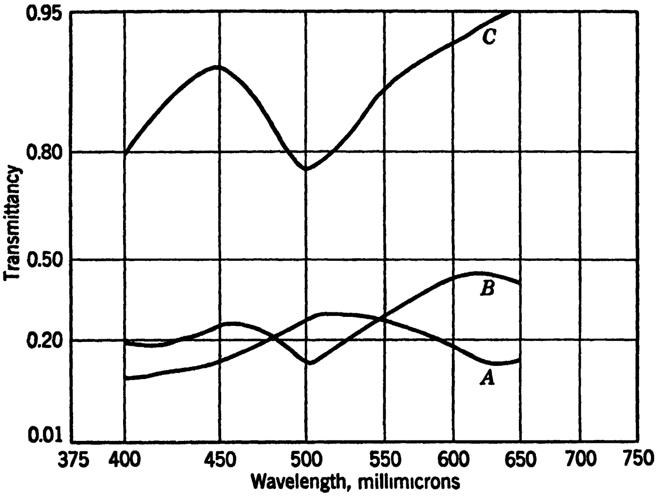

FIG. 7·64. Curves for color test for vitamin D in presence of provitamins.

A = calciferol;
B = ergosterol;
C = 7-dehydrocholesterol.

Solvent = chloroform; ratio of k_s at 625 mμ for A, B, C = 100, 4, 0.3, respectively; see Sobel *et al.* (*181*).

Sobel and coworkers (*181*) have proposed a color test to measure combined D_2 and D_3 and to distinguish the provitamins. The color is developed by addition of glycerol dichlorohydrin in the presence of acetyl chloride and mixture of reagent with solvent. The colors obtained vary, depending upon the amount of acetyl chloride added and the reagent-solvent ratio. Suitable conditions for the estimation of calciferol in the presence of ergosterol and 7-dehydrocholesterol are 1 per cent acetyl chloride in glycerol, 1,3-dichlorohydrin with chloroform as a solvent, and a solvent-reagent ratio of 3 to 2. The absorption spectra under these conditions are shown in Fig. 7·64, and it is evident that little interference is encountered at 625 mμ for the estimation of calciferol. By changing the conditions to 4 per cent acetyl chloride and a solvent-reagent ratio of 2 to 3, the absorption spectra shown in Fig. 7·65 are obtained, and it is evident that ergosterol can be determined at 500 mμ with very little interference. In cases like this it might be desired to set up a two-component analysis based upon these two different conditions and two different wavelengths. This illustrates the point that a two-component analysis at two wavelengths need not be restricted to a single solution.

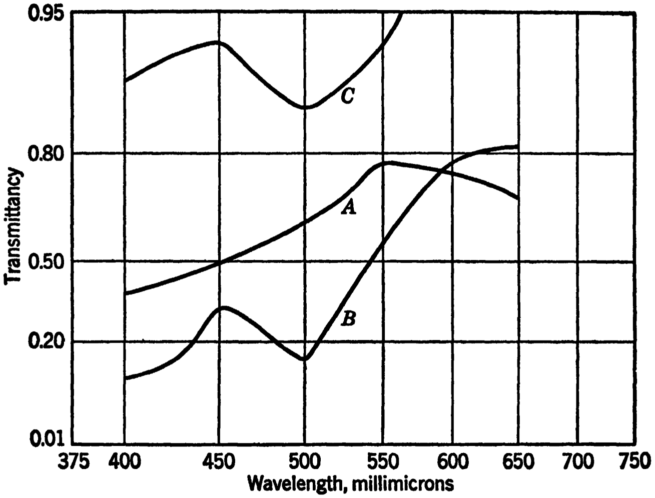

FIG. 7·65. Curves for the color test to distinguish ergosterol from 7-dehydrocholesterol.

A = calciferol;
B = ergosterol;
C = 7-dehydrocholesterol.

Solvent = chloroform; ratio of k_s at 500 mμ for *A*, *B*, *C* = 100, 44, 3, respectively; see Sobel *et al.* (*181*).

Other colorimetric reactions given by vitamin D have been reviewed by Ewing *et al.* (*69*) and Lamb *et al.* (*117*).

7·64 Hormones. The analytical application of spectrophotometry to hormones has been limited by the fact that many hormones do not possess absorption spectra which are characteristically different from inactive compounds. The greatest usefulness of the method has been in the measurement of pure hormones to assist in structure determination. The absorption spectra of three steroid hormones are shown in Fig. 7·66.

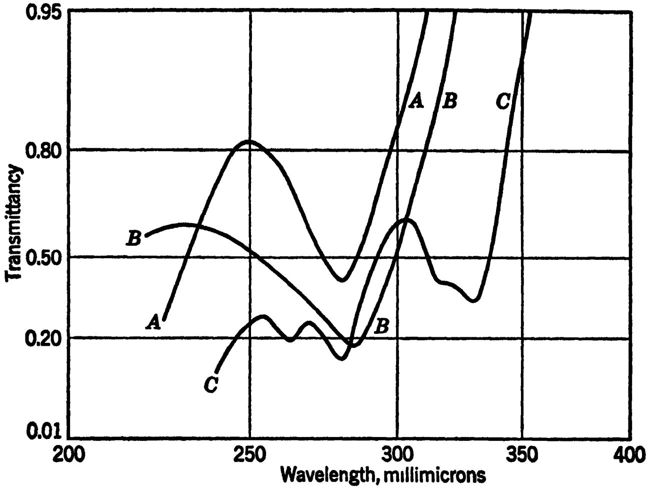

FIG. 7·66. Absorption spectra of some hormones.

A = estrone;
B = androsterone;
C = equilenin.

k_s = 8000 for *C* at 280 mμ; see Brode (*33*).

7·65 Enzymes. Absorption spectra have been primarily of use in qualitative analysis of structure. As an example, the porphyrin part of the cytochrome c molecule is believed to give rise to the characteristic absorption spectra (*195*). Because the reduced and oxidized forms of cytochrome have different spectra, it is believed that it is the porphyrin part that is involved in oxygen transfer. This is shown in Fig. 7·67.

7·66 Clinical applications. Colorimetric methods are extensively used in clinical tests in order to determine various compounds of physiological importance in blood and urine. Much of the routine work is done with colorimeters or filter photometers rather than with spectrophotometers. However, in those cases where spectrophotometric studies have been made the additional information concerning the identity and quantity of foreign substances and the stability of the compound in question has been valuable. The work of Drabkin illustrates this point. Mixtures of methemoglobin and oxyhemoglobin have been analyzed for relative amounts of each, and the total determined by converting both to cyanohemoglobin (*58*). The conversion of nitric oxide hemoglobin to methemoglobin and the formation of

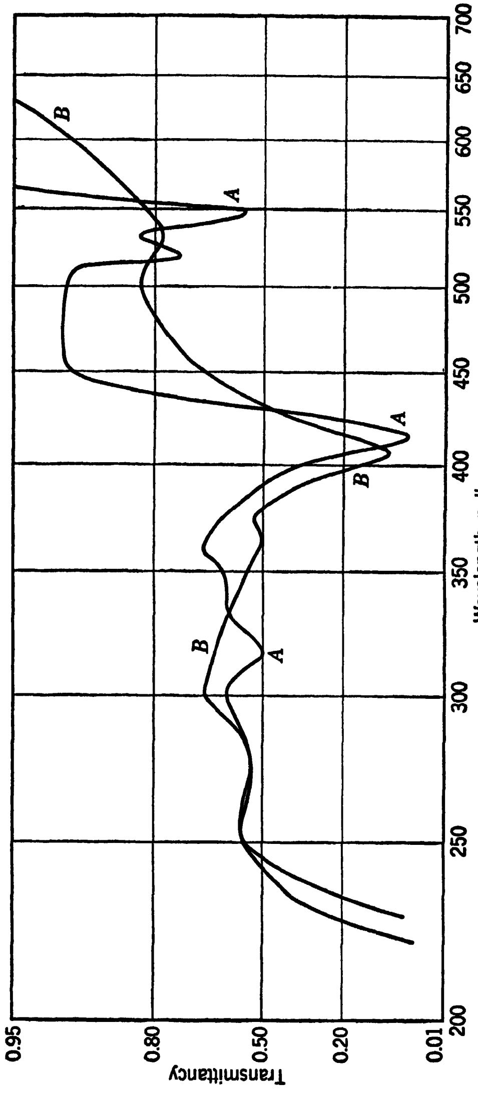

Fig. 7·67. Absorption spectra of cytochrome c.

A = reduced form;
B = oxidized form.

k_s = 367 at 415 mμ for *A*; see Theorell (*195*).

sulfhemoglobin have been followed (*59*). Methemoglobin (*8*) and the hemochromogens (*60*) have been extensively studied. Some of this work involved the use of a special solution cell only 0.07 mm. thick which could be used to measure undiluted blood without letting it come into contact with air (*61*). The circulating blood of a live dog has been measured (*62*). Figure 7·68 shows the curves of oxy- and methemoglobin at the same concentration. If the blood of a patient contains some of each, as in cyanosis, the relative percentage of methemoglobin may be read from the scale shown in the figure after the curves have been matched at the wavelengths where the curves cross. It has been proposed that total pigment be determined as cyanomethemoglobin, which has a molar absorbancy index of 11.5 at 540 mμ (*57*).

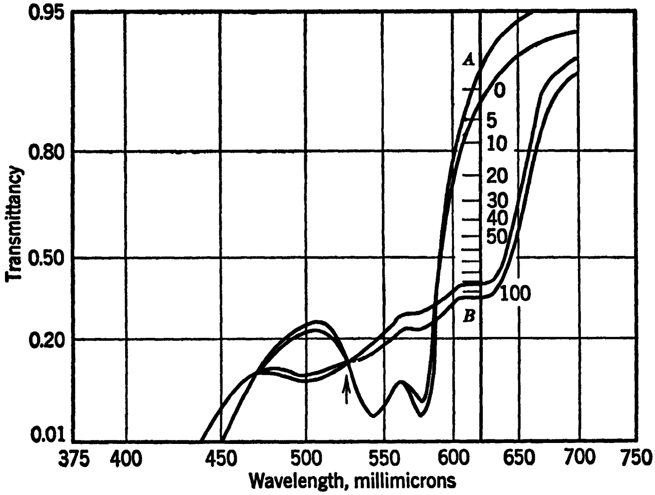

FIG. 7·68. Curves for the determination of methemoglobin in venous blood.

A = oxyhemoglobin showing variation in 25 individuals;
B = methemoglobin showing variation.

Arrow indicates wavelength to superimpose curve of unknown sample; scale indicates relative percentage of methemoglobin. Solvent = water; concentration arbitrary; see Hamblin and Mangelsdorff (*83*).

Glucose in blood or urine may be determined by a redox reaction which takes advantage of the reducing power of glucose. If the material reduced is cupric sulfate, the cuprous oxide which is formed is reacted with molybdiphosphoric acid, which in turn is reduced to molybdenum blue which may be measured spectrophotometrically (*89*). If the material reduced is potassium ferricyanide, it becomes the colorless ferrocyanide, and the loss in yellow color may be measured spectrophotometrically (*89*). This same color reaction will also measure ethyl alcohol in blood and urine (*75*).

The total nitrogen content may be determined with a micro-Kjeldahl technique followed by color development with Nessler's solution (*91*). Total protein, albumin, and globulin may be estimated by the color developed in the biuret reaction (*111, 132*).

Urea may be determined by converting it into ammonium carbonate by the action of the enzyme urease, removal of interfering blood proteins and urease with zinc sulfate and sodium hydroxide, and development of the yellow color with Nessler's reagent (*91*). Urea may also be determined by the red color developed when it is heated in acid with alpha-isonitrosopropiophenone (*6*).

Uric acid may be determined by the blue color developed by reducing tungstiphosphoric acid (*39*) or arsenotungstiphosphoric acid (*40*). A number of other substances normally present in blood also develop the color. This interference may be overcome by making two determinations, one on the original blood, and a second on a blood sample which has been reacted with the enzyme uricase. The difference in the developed color of these two solutions is due to uric acid (*16*).

The Jaffe reaction, a test for creatinine, is carried out by reacting the sample of blood or urine with alkaline picrate, which develops a red color (*17*). The test can be made specific for both creatine and creatinine by exposing one of two samples to the reaction of adaptive bacterial enzymes which specifically destroy creatinine (*3*).

Cholesterol in blood may be determined by the blue color developed in the Lieberman-Burchard reaction (*183*). Vitamin A and carotene in human blood may be determined (*210*).

Bilirubin determined by its own yellow color is subject to error because of interfering colors such as those of the carotenes (*128*). It may be estimated by the van den Bergh reaction, which consists of making a red azo dye with it and sulfanilic acid (*65, 174*).

The above examples are merely illustrative of the possibilities of analytical spectrophotometry in clinical work. Other examples may be found in more comprehensive treatments of the subject (*90*).

7·67 Aromatic hydrocarbons. Although the prediction of the absorption spectrum of a particular compound from a knowledge of its chemical structure is in general impossible with the present state of knowledge, and conversely the determination of structure from absorption spectra cannot be rigidly applied, a large amount of empirical knowledge has been accumulated which is of considerable value. The aromatic hydrocarbons have been reviewed by Jones (*104*). Each of the unsubstituted hydrocarbons of the type benzene, naphthalene, anthracene has a characteristic absorption spectrum. The characteristic natures of these spectra are best brought out by plotting on a log A_s basis, so that concentration changes will not introduce curve changes. Two of these are shown in Fig. 7·69.

If these basic unsubstituted hydrocarbon chromophore groups are present in a molecule in such a manner that they do not mutually resonate with each other, as may be accomplished by having them separated by a methylene group, then the effect of each is essentially independent. Figure 7·70 shows the curves of ethylbenzene, bibenzyl, and triphenylmethane, which contain in their molecular structure 1, 2, and 3 benzene rings, respectively. These benzene rings are isolated from each other, and hence, when plotted on an equimolar basis, their spectra would be expected to be closely parallel and separated vertically. Thus bibenzyl would be expected to have twice the absorption of ethylbenzene and hence on a log A_s plot should be vertically separated from it at all wavelengths by log 2, which is 0.3. Similarly, triphenylmethane would be expected to have three times the absorption of ethylbenzene because its molecule has three times as many benzene ring chromophores. Its absorption spectrum should therefore be vertically displaced by log 3, which is 0.5. Figure 7·70 shows that this is roughly approximated.

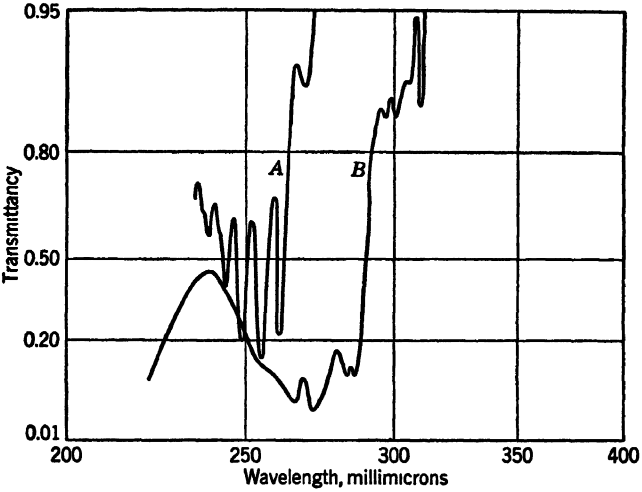

FIG. 7·69. Absorption spectra of aromatic hydrocarbons.
A = benzene;
B = naphthalene.
Concentration of $A = 10B$; solvent = cyclohexane; $a_s = 2.95$ at 255 mμ for A; $a_s = 47$ at 272 mμ for B.

The substitution of an alkyl group for a hydrogen atom attached to a carbon atom of an aromatic hydrocarbon chromophore changes the curve shape only slightly. The general effect is a bathochromic shift. In a study of 1,2-benzanthracene derivatives (*104*) 25 alkyl and alicyclic derivatives were measured and the effect on the absorption curve noted. The parent compound has twelve absorption maxima between 240 and 390 mμ, and all 25 of the derivatives showed from 9 to 13 maxima. The positions of two of the more prominent maxima are 287 and 341 mμ on the parent compound. These were shifted various amounts in the various derivatives, the greatest observed variation being 287 to 298 mμ and 341 to 364 mμ, respectively. The absolute value of the molar absorbancy index varied, the total range being less than 2 to 1. In one sense the curves were all different, since the number, position, and magnitude of the absorption maxima changed in each compound. Tables of wavelength position of maxima would not serve the purpose of identification. On the other hand, in all the derivatives the general character of the absorption spectrum of the parent hydrocarbon is maintained. Similarity and differences between spectra of two compounds can be discerned most readily if the plots are made on trans-

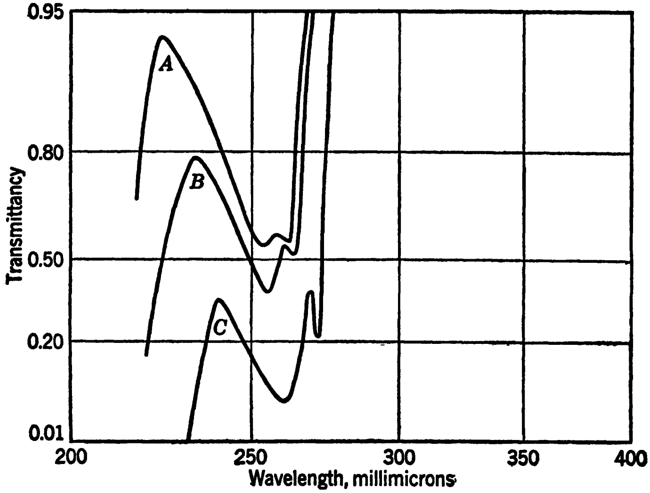

FIG. 7·70. Curves showing additive effect of isolated chromophore groups.

A = ethylbenzene (1 benzene chromophore);
B = bibenzyl (2 benzene chromophores);
C = triphenylmethane (3 benzene chromophores).

All solutions at equimolar concentration; solvent = ethanol; a_s = 3.65 at 361 mμ for *C*; see Jones (*104*).

parent paper with a log A_s ordinate, so that they may be superimposed, adjusted vertically to compensate for differences in concentration of chromophore group, and compared.

On the other hand, if the basic hydrocarbon chromophore groups can resonate with each other through their linkage, or if substitution is made on the chromophore group by an aryl or alkene group that can resonate with it, then the spectrum is radically changed. This is illustrated in Fig. 7·71, where the spectrum of ethylbenzene is changed radically by introducing unsaturation in the side chain.

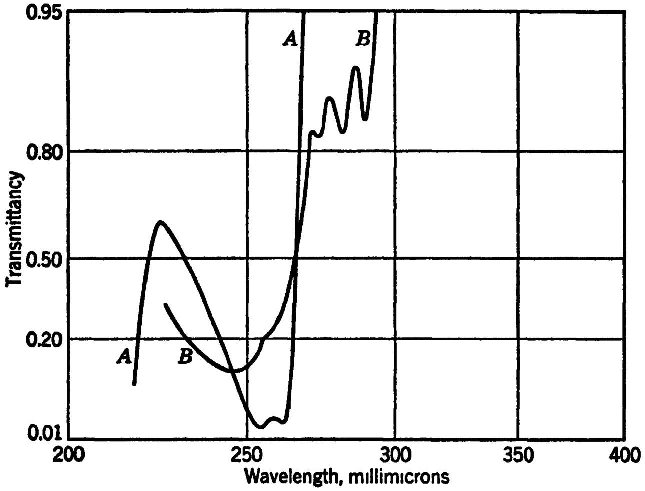

FIG. 7·71. Curves showing structural effects.

A = ethylbenzene;
B = styrene.

Solvent = ethanol; molar concentration of $A = 0.01B$; $a_s = 144$ at 247 mμ for B; see Jones (*104*).

For information on resonating and insulating linkages of chromophore groups, reference may be had to books that deal with the theory of the origin of absorption spectra (*31*, *34*). From the viewpoint of qualitative identification three points should be stressed. Identity of curve shape is evidence for identity of the chromophore part of the molecule only, not of the entire molecule. Similarity of curve shape rather than identity may be cited as evidence of structure. Introduction of a resonant substituent group is effectively a change in the chromophore.

7·68 Chlorophylls. Spectrophotometric analyses of mixtures of chlorophyll a and b have been carried out (*212*). Figure 7·72 shows the absorption spectra of these two compounds. If it is desired to determine total chlorophyll, it is possible to make a single measurement at a wavelength where the two compounds have identical absorbancy indexes. Such a point is 440 mμ.

Analyses have also been carried out for mixtures of chlorophyll a, chlorophyll b, leaf xanthophyll (actually a mixture but considered as one component), and beta-carotene (*81*, *139*). The solutions used were ether extractions of plant tissues of corn and barley. Analyses of this type have been useful in plant-pigment inheritance studies (*100*, *101*, *102*). In the study of inbred corn the total carotenoids and total chlorophyll of young plants extracted with acetone and measured in ether were determined. Also the total carotenoids and beta-carotene in the endosperm of the adult plant were determined. These quantitative analyses were correlated with factors of biological importance.

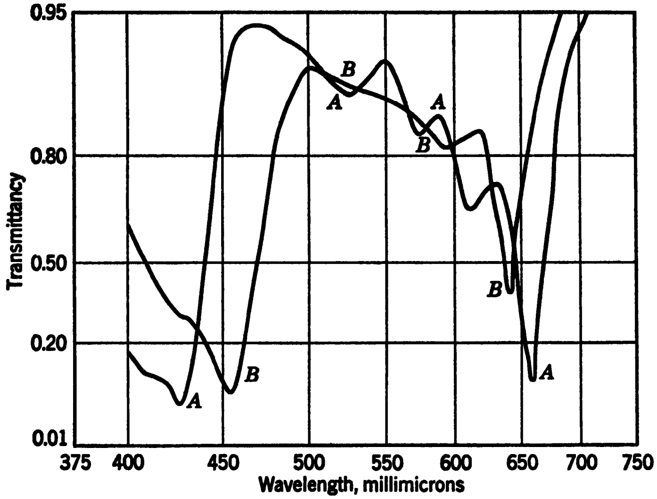

FIG. 7·72. Absorption spectra of chlorophylls.
A = chlorophyll a;
B = chlorophyll b.
Solvent = diethyl ether; a_s = 102 at 425 mμ for A; see Zscheile (*212*).

7·69 Dyes. Spectrophotometry is widely used in the research, manufacture, and application of dyes. The relation between structure of dyes and color has been the subject of study in several laboratories (*38*, *88*, *122*, *123*, *166*). The physical and chemical nature of dyes in solution has been studied (*124*, *165*). In general, the tendency has been for one investigator to limit his treatment to one class of dyes, and usually the relation between structure and color has been limited to a discussion of the effect of variations in a parent molecule on the color. In spite of the empirical nature of this approach, much information has been obtained which is of value in the prediction of color of yet unsynthesized dyes.

In the manufacture of a dye the spectrophotometer may be used to determine the purity. The commercial dye naphthol blue black (C.I. 246) has been studied by Appel *et al.* (*5*). It was found that samples of this commercial dye from many manufacturers all contained various

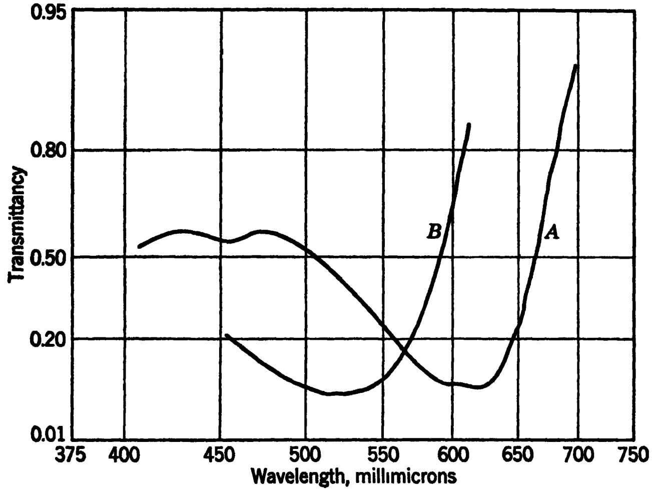

FIG. 7·73. Curves illustrating the determination of purity of the dye naphthol blue black (C.I. 246).

A = commercial naphthol blue black;
B = nonsubstantive impurity.

Concentrations arbitrary; solvent = water; see Brode (*29*).

impurities which in general were red, the color of the dye itself being blue. The presence of these impurities was found largely by the fact that, when wool was dyed with the commercial dyes, these red impurities tended to concentrate in the dye bath. In a sense the wool was used as an adsorbant in a chromatographic method of separating components. Figure 7·73 shows the curves of a typical commercial dye of this type and the red impurities nonsubstantive on wool. Although the relation of structure to color is not established well enough to infer the structure of these impurities from their absorption curve, it is possible to synthesize probable impurities, and when a probable impurity is found which has a similar absorption curve the identity of the impurity is inferred (*29*). The problem is further complicated by the fact that the red "impurity" is likely to be a mixture of several by-products.

In attempting to purify a particular dye the course of the purification may be followed spectrophotometrically. Two evaluations which are useful in this connection are the apparent concentration and the impurity index. Figure 7·74 illustrates this process. The first material prepared may be taken as an arbitrary standard and assigned the arbitrary purities of 100 and (I.I.) = 0. The dye is then subjected to recrystallization or some other purification process, and the purity determined by the method of Section 7·36 and the impurity index by the method of Section 7·51. In the example of Fig. 7·74 the second measurement shows a purity of 110 per cent and an (I.I.) of −0.1. The gain in apparent purity from 100 to 110 indicates the removal of a material which absorbs less strongly at the wavelength of purity determination. The reduction in the impurity index means that a colored impurity has been removed. ' If the apparent concentration increases with no change in the (I.I.), it means that the impurity being removed is a colorless diluent such as salt or moisture. If the (I.I.) is reduced but the apparent purity unchanged, it indicates the removal of a colored impurity which absorbs at the wavelength of purity determination about as much as does the desired dye and also absorbs at the wave-

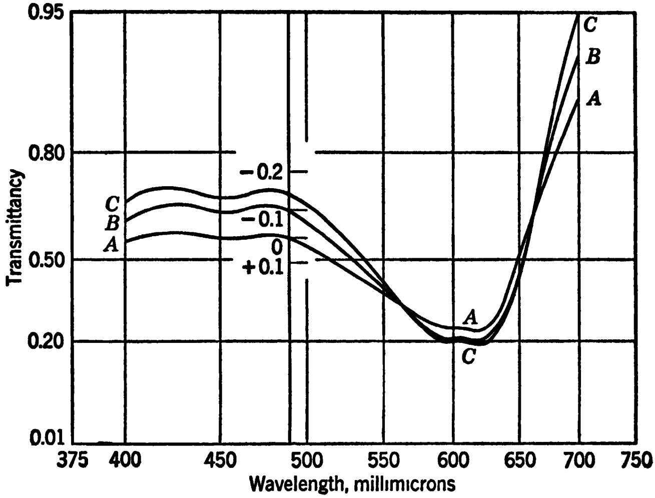

FIG. 7·74. Curves showing the effect of purification of a dye.

A = impure dye;
B = dye after one recrystallization;
C = dye after two recrystallizations.

Scale at 490 mμ is an impurity index scale; relative concentrations of dye are read at 620 mμ.

length of the (I.I.) to a greater extent than the dye. After a second treatment, the apparent purity of the dye of Fig. 7·74 increased to 113 and the (I.I.) reduced to −0.15. When further treatment fails to increase the apparent purity or to reduce the impurity index, there is no need to proceed further by that method. This material may then be set up as a new standard and assigned arbitrarily a purity of 100 per cent and (I.I.) = 0. This standard may then be used for reference in subsequent analyses. It is possible for a subsequent sample, perhaps purified by another method, to show a greater apparent purity or lesser (I.I.).

In the determination of purity of a dye chemically it is possible to estimate the actual purity. Thus for an azo dye which may be titrated with titanium trichloride, which depends upon the reduction of the azo group, it is possible to write the chemical equation involved and to determine stoichiometrically the amount of titrating reagent required for a 100 per cent pure sample. The observed result of a particular analysis can be divided by the theoretical amount and the purity estimated. This differs from the spectrophotometric method, which always uses a reference material as arbitrary standard. If the relation between structure and color were completely worked out, it would be possible to predict the absorbancy index of the pure dye, and then the spectrophotometric purity could be put on an absolute rather than on a relative basis. These relationships are not yet worked out, and hence absolute spectrophotometric purities must depend on other methods of analysis.

The spectrophotometric determination of purity of a dye may be thrown in error by the presence of absorbing compounds other than the desired component. This possibility is of course minimized by determining the (I.I.), as well as the purity, versus the standard. The chemical determination of purity of a dye may be thrown in error by the presence of titratable impurities. Therefore, neither method is infallible.

Many commercial dyes are not pure. Some impurities may arise by simultaneously occurring side reactions in the manufacture. Many dyes are made in water solution, and in order to precipitate them it is necessary to add salt. Some of the salt precipitates with the dye. Thus, both colored and colorless impurities are likely to be present. In addition, many dyes are hygroscopic and absorb atmospheric moisture. Commercial standards are based on these impure dyes, and formulas in the various mills which produce dyed material are based on certain amounts of these standards per unit weight of material to be dyed. It is therefore necessary for the dye manufacturer to standardize his products. The usefulness of the spectrophotometer in dye standardization is generally appreciated (*63*, *64*, *77*, *149*, *162*, *167*, *173*, *175*, *186*, *202*) and is recognized in some government specifications (*197*, *198*).

When several dye manufacturers offer similar products, mills often prepare dyed samples, using the various available standards, and choose the manufacturer that offers them the best money value, that is, the dye with the greatest purity apparent visually. There is some randomness involved here in the preparation of the dyed sample and the visual estimation of purity. To avoid these sources of randomness it has been proposed to evaluate competitive dyes with solution measurements (*5*). Such measurements are accompanied by certain difficulties in correlating instrumental data obtained with solutions with visual data obtained with dyeings (*186*). One factor which affects the correlation is the variation in nonsubstantive absorbing materials. A second factor is the difficulty of estimating apparent visual purity from spectrophotometric data. In the rare case where the competitive products have identical purities at all wavelengths, as determined by the ratio of the absorbancy indexes, the results at any one wavelength will correlate well with visual purity, other factors being equal. But the usual case is to have varying spectrophotometric purities determined at various wavelengths, and the problem is to choose the wavelength which will

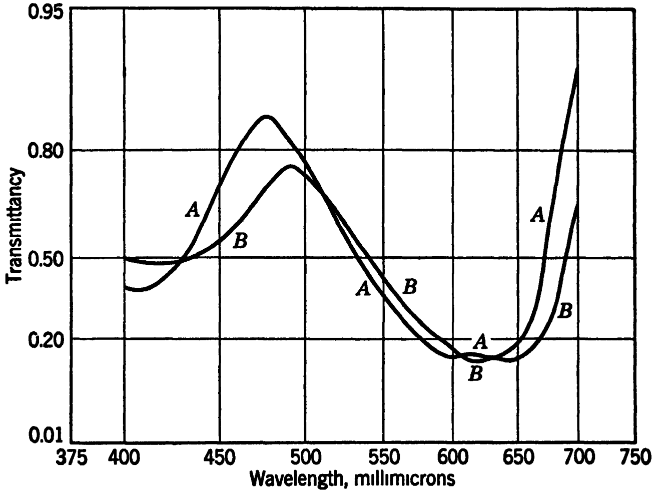

FIG. 7·75. Curves illustrating dye identification by curve shape.

A = alizarin cyanine green (C.I. 1078);
B = brilliant benzo green GL.

Solvent = water; these dyes have visually similar chemical spot tests; see Stearns (*186*).

correlate with the visual. A third factor is that the dyes may vary in the efficiency (*168*) with which they color a substrate. Despite these difficulties of correlation the method is used.

Another application is in dye identification. For single products two general methods are available, curve shape and color reactions. Figure 7·75 illustrates identification by curve shape. These two dyes have visually similar color reactions with the various reactive chemicals commonly used for test (*47*, *78*), but they are readily differentiated by curve-shape differences. Figure 7·76 illustrates identification by color reactions. In water solution the xylene light yellow has a curve shape similar to that of other yellow dyes, but in alkaline solution it has a markedly different color which serves to characterize it.

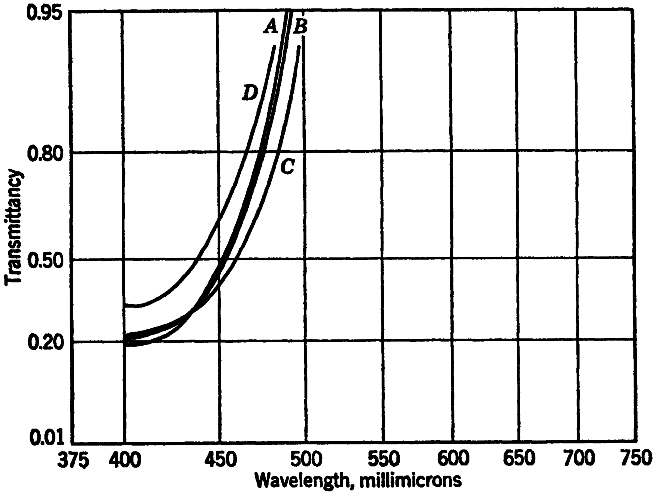

FIG. 7·76. Curves illustrating dye identification by reactive solvents.

A = xylene light yellow (C.I. 639) in water;
B = fast light yellow (C.I. 636) in water;
C = xylene light yellow in 10 per cent caustic solution;
D = fast light yellow in 10 per cent caustic solution.

Note that the two yellow dyes which are rather similar in water, curves *A* and *B*, are readily distinguished by the curves in a 10 per cent sodium hydroxide solution, curves *C* and *D*.

The spectrophotometric curves of the exhaust liquors which remain in the dyebath are sometimes very instructive (*186*). When a certain sample of alizarin saphirol B (C.I. 1054) was dyed and the exhaust measured, the initial solution was found to have a curve as shown by curve *A* of Fig. 7·77. The exhaust after a short period of time was found to have curve *B* of Fig. 7·77. Curve *A'* of Fig. 7·77 shows curve *A* displaced vertically. If the dyeing were merely the exhaustion of a pure color, curves *A* and *B* should be identical in shape within the limits of Beer's law, which is obeyed very closely over such small concentration changes. The fact that they were different indicated that the dye was not pure, and it was suspected that there was an impurity or toning color which exhausted more rapidly than the main color. When curve *B* was subtracted from curve *A'*, by the method of Section 7·4, curve *C* was obtained. Curve *C* plotted on a log A_s ordinate has a characteristic curve shape which made it possible to identify it. The curve shape agrees exactly with that of victoria fast violet 2REx (Pr. 197).* It was therefore evident that the violet was added to the blue as a shading color to alter its color slightly.

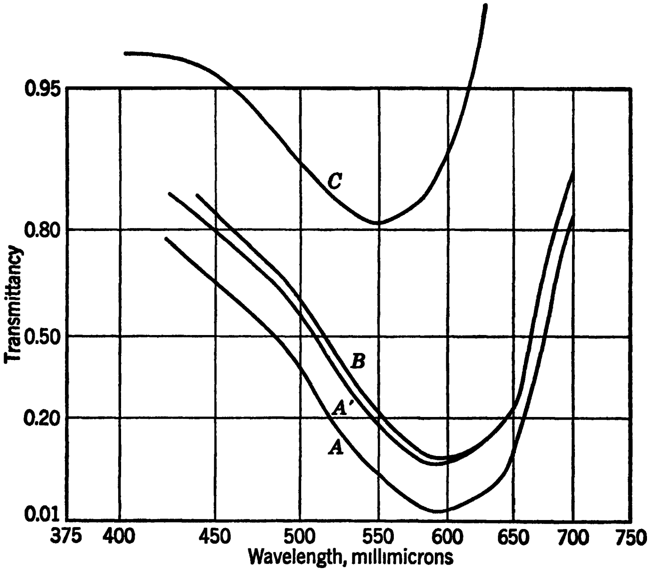

FIG. 7·77. Curves for the analysis of a dyebath exhaust, including the detection of a toner.

A = alizarin saphirol B, initial dyebath;
A' = curve *A* displaced for comparison;
B = exhaust solution after short time;
C = calculated toner, victoria fast violet 2REx.

Solvent = water; see Stearns (*186*).

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* A dye identification number used in the yearbook of the American Association of Textile Chemists and Colorists.

Measurements of exhausts also give evidence of chemical identity and the presence of impurities (*186*). For instance, in Fig. 7·78 curves *A* and *B* represent two samples of an acetate blue and show that there is a definite chemical difference between the two samples. It is impossible to tell from the curve shapes alone whether there is a fundamental difference in the molecular structure of the two dyes or whether impurities are present which are responsible for altering the curves from that of the pure dye. A comparison of the exhausts of these two colors after they had been dyed showed that the dye with curve *B* had a red nondyeing impurity present in it which was not present in *A*. The exhaust curves are *C* and *D* of Fig. 7·78. The exhausts were measured at a suitable concentration and cell length to fall in the range of transmittancies for accurate measurement, but have been traced on Fig. 7·78

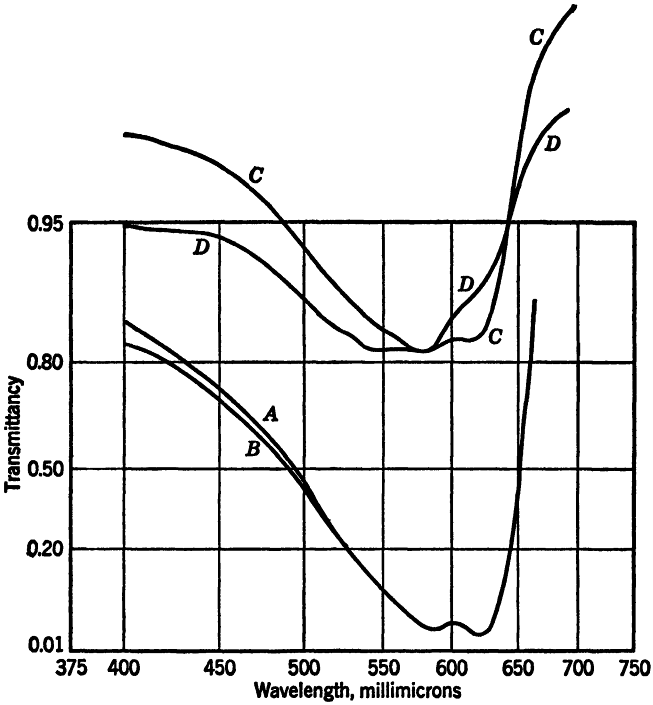

FIG. 7·78. Curves for exhaust analysis, including the detection of an impurity in acetate blue.

A, *B* = initial solutions;
C = exhaust of *A*;
D = exhaust of *B*.

See Stearns (*186*).

vertically displaced, so that the concentrations of solutions *A*, *B*, *C*, and *D* are all comparable and the graphical method of analysis described in Section 7·4 may be applied. When the exhaust curves were subtracted from the curves of the initial dyes, the two resulting curves were identical. This indicated that the same dye had gone into the fiber in both cases. The exhaust analysis has thus shown that the dyes were essentially chemically identical but that *B* contained some impurities in small amount.

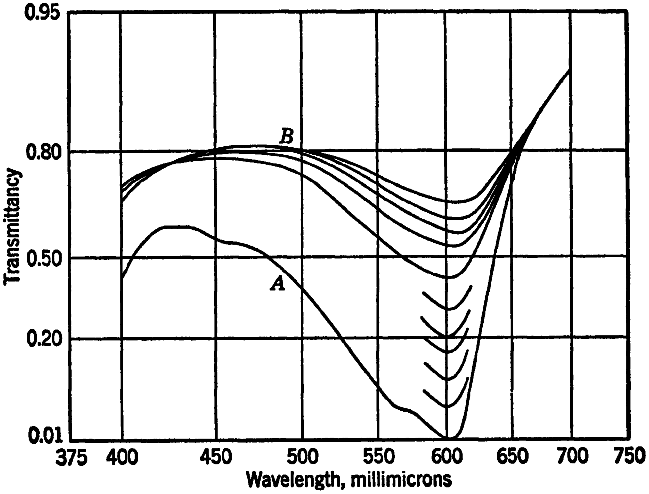

FIG. 7·79. Curves showing exhaustion of caledon jade green (C.I. 1101).

A = initial dyebath;
B = final dyebath.

See Stearns (*186*).

The spectrophotometer has also proved useful in studying the application properties of dyes. One application property of importance is the rate of exhaustion. It is evident from the foregoing discussion that it is desirable to obtain the spectrophotometric curve over a large range of wavelengths at each interval of time at which it is measured, as this permits the detection of unsuspected toners and impurities. This can be done with slow-exhausting colors, but with fast-exhausting colors it is possible only to run a portion of the curve. The exhaustion of caledon jade green (C.I. 1101) is shown in Fig. 7·79. In the early part of the exhaustion the reaction is so rapid that only a small section of the curve may be run. During the later portion of the dyeing the entire curve may be run. When the concentrations are determined, a plot of concentration versus time can be obtained, and these are the familiar exhaustion curves (*44, 143, 144, 168*). Where the exhaustion is extremely rapid, the spectrophotometer can be adapted to plot transmittancy directly as a function of time (*186*). Figure 7·80 shows the spectrophotometric curves of a dyebath before and after reduction, curves *A* and *B*, and also the time curve of the transmittance at 610 mμ drawn directly by the instrument, curve *C*.

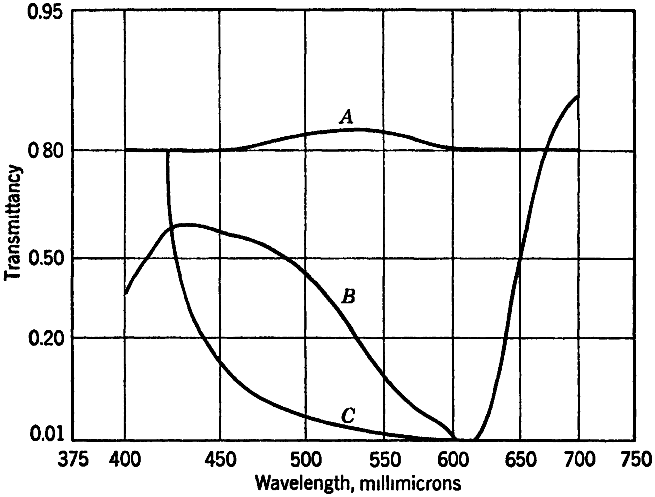

Fig. 7·80. Curves showing rate of reduction of caledon jade green.

A = initial dyebath;
B = final dyebath containing leuco form of the dye;
C = a time plot of transmittance at 610 mμ drawn directly by the spectrophotometer.

See Stearns (*186*).

Another application problem of interest is the fastness of dyes, that is, their ability to maintain their original color in use. The relation of structure to fastness to light and to fastness to washing has been studied (*79, 80, 110*).

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8· SPECTROPHOTOMETERS:

Infrared Region

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8·1 Introduction. Although such distinguished scientists as J. F. W. Herschel, Young, Beer, Lambert, Melloni, Rubens, Boys, Fizeau, Foucault, Kirchhoff, Langley, Talbot, Paschen, Ångström, Seebeck, and Julius contributed to the solution of the many and varied experimental difficulties associated with infrared spectrometry, it was Julius who first pointed out the analytical possibilities of the new science. In 1892, he (*31*) published a paper in which he demonstrated the relation between the structure of organic molecules and their infrared spectra. He showed empirically that the presence of a methyl group in a molecule gave rise to characteristic absorption that appeared to be independent of the constitution of the rest of the molecule. Similar observations were made for amino, hydroxyl, aromatic, and other functional groups. The conclusions of Julius were confirmed and extended by the monumental work of W. W. Coblentz (*16*), which was carried out over a period of some years, and was first published by the Carnegie Institution of Washington in 1905. His early works, published in three volumes, not only discussed instruments and experimental techniques, but also gave the absorption and reflection spectra of a large number of organic and inorganic materials.

Because of the impossibility of making precise measurements with the existing equipment, the poor resolution of the spectrometers, and the difficult techniques involved in the operation of the early instruments, the analytical possibilities of the new science received little attention. It is not surprising, therefore, that the next quarter of a century was devoted primarily to the problems of instrumentation and the theoretical aspects of infrared spectroscopy. During this period, such devices as the echelette grating for the near infrared and wire gratings and reststrahlen spectrometers for studying the far infrared were introduced. The use of curved slits and of off-axis parabolas as collimators became more or less common practice. Vacuum thermocouples with especially high thermoelectric power and galvanometer amplifiers were innovated. When instrument manufacturers placed modern, high-sensitivity galvanometers on the market, infrared spectroscopy was stimulated enormously. Successive contributions made it possible to construct instruments having higher resolution, greater stability, and greater range than were previously possible.

As better instruments became available, infrared spectroscopy as a research tool for the study of organic structure and for the analysis of chemical mixtures again came into prominence.

8·2 Origin of infrared spectra. In any molecule not at absolute zero, the atoms are constantly oscillating about their respective equilibrium positions with frequencies corresponding to the infrared portion of the spectrum. If, during these oscillations, a change in the dipole moment of the molecule occurs, a periodically changing electric field will be produced around the molecule. As a result, emission or absorption of radiant energy at the oscillating or vibrating frequencies will occur. Diatomic molecules, such as hydrogen, oxygen, and nitrogen, do not emit or absorb because the vibration of the atoms of such molecules produces no changes in their dipole moments. However, a molecule that has no permanent dipole may emit or absorb radiant energy if, when it vibrates, a dipole is produced. Thus, carbon dioxide, although it has no permanent dipole, absorbs and emits radiant energy at frequencies corresponding to the asymmetrical vibrations of the molecule.

If the vibration of a diatomic molecule is purely harmonic, and if the dipole moment is a strictly linear function of the displacement from the equilibrium position, then the electric moment will behave as a harmonic oscillator, and the vibrational energy associated with the molecule is given by wave mechanics as,

$$E_{\text{vib.}} = (n + \tfrac{1}{2})h\nu_0 \qquad (8\cdot 1)$$

where n is the vibrational quantum number ($n = 0, 1, 2, \cdots$), h is Planck's constant, and ν_0 is the fundamental vibrational frequency. On the other hand, if the vibration is anharmonic (the dipole moment is not a linear function of the displacement), or if the vibration is not strictly harmonic, the electric moment will not behave as a harmonic oscillator. In this case the absorption spectrum will contain absorption frequencies corresponding to the fundamental vibration and to overtones as well. The two causes which give rise to overtones are the anharmonicity of the vibration itself, and the nonlinear change of the dipole moment with atomic displacement. These are called, respectively, the mechanical anharmonicity and electrical anharmonicity.

If the vibrations of a polyatomic molecule are strictly harmonic, and if the displacement of the dipole moment depends in a strictly linear manner on the atomic displacements, then the normal vibrations and the changes of dipole moment produced by the normal vibrations will be independent of each other. The result is that the vibrational spectrum will contain not only frequencies corresponding to the fundamental vibration of the entire molecule, but also frequencies associated with the vibrations of the substituent groups of the molecule. In the ideal case, the frequencies will be independent of each other; however, mechanical or electrical anharmonicity usually is present, and overtones result. Moreover, there may appear the sums and differences of fundamentals or overtones. These are called combination frequencies. It may happen that a combination frequency will appear even though the fundamental frequencies have zero intensities.

If, when the molecule absorbs or emits radiant energy, only the rotational energy is affected, the change per quantum absorbed by diatomic molecules being given by the equation,

$$\Delta E_{\text{rot.}} = h\nu \qquad (8\cdot 2)$$

It can be shown that,

$$\nu = \frac{h^2 m(m+1)}{8\pi^2 I} \qquad (8\cdot 3)$$

where I is the moment of inertia of the molecule about its axes of rotation, and m is the rotational quantum number. In this expression m is the only variable, and according to wave mechanics it may change only by a single unit, that is, $\Delta m = \pm 1$.

Thus, the lines making the rotational spectrum will have frequencies given by

$$E_{\text{rot.}} = \frac{2mh}{8\pi^2 I} \qquad (8\cdot 4)$$

Since m can have values 1, 2, 3, $\cdots$, the lines will be separated by constant frequency intervals $2h/8\pi^2 I$, usually expressed in terms of wave numbers.

From the observed separation of the lines the moment of inertia, and hence the dimensions of the molecule, can be deduced. Since I is usually very large relatively, the rotational spectrum occurs in the far infrared, where it is very difficult to make observations. In addition, molecules which do not possess a permanent dipole do not exhibit rotational spectra. For nonpolar molecules the moment of inertia can sometimes be obtained from Raman data.

When both rotational and vibrational energies change simultaneously, absorption occurs in the near infrared. The frequencies of the bands making up the rotational-vibrational spectrum are given by the equation,

$$\nu = \Delta(E_{\text{rot.}} + E_{\text{vib.}})\,\frac{1}{h} \tag{8·5}$$

Adding the rotational and vibrational energy expressions, one obtains

$$E_{\text{rot.}} + E_{\text{vib.}} = \frac{h^2 m(m+1)}{8\pi^2 I} + (n + \tfrac{1}{2})h\nu_0 \tag{8·6}$$

Hence, for a simultaneous transition from the vibrational state, n_1 to n_2, and the rotational transition m_1 to m_2,

$$\Delta E = \Delta(E_{\text{rot.}} + E_{\text{vib.}}) = h\nu \tag{8·7}$$

$$\nu = \frac{h[m_2(m_2+1) - m_1(m_1+1)]}{8\pi^2 I} + \nu_0(n_2 - n_1) \tag{8·8}$$

Since $\Delta m = \pm 1$, and for the fundamental vibrational band, $n_2 - n_1 = 1$, we can write

$$\nu = \nu_0 + \frac{2hm}{8\pi^2 I} \tag{8·9}$$

The first term in this expression fixes the position of the center of the vibration band, while the second term determines the rotational fine structure separation in it. The moment of inertia I can be deduced from the observed separation in bands which lie in a region of the spectrum which is much more accessible than that of the pure rotation spectra.

An interesting feature of the rotational-vibrational spectrum is that for a diatomic molecule whose moment of inertia about a line joining the nuclei is zero, m may have values of ± 1, ± 2, ± 3, $\cdots$, but never 0. As a result, a band of two branches is formed in which the fundamental frequency is not observed. This is illustrated with the infrared spectrogram of HCl gas shown in Fig. 8·1. When m in the equation,

$$\nu = \nu_0 + \frac{2hm}{8\pi^2 I}$$

is positive, a series of lines lying on the higher frequency side of ν_0 is obtained. These lines form what is known as the R-branch of the band. Similarly, for negative values of m, a P-branch at lower frequencies is formed. With such molecules as NH_3, in which the value $m = 0$ is permitted, a group of lines about the fundamental frequency ν_0 forms the Q-branch.

The presence of the Q-branch always indicates the possibility of the molecule's possessing angular momentum about an axis joining the nuclei. This is due to the fact that the component of the dipole moment along the axis of rotation (the latter being perpendicular to the axis joining the nuclei) is not influenced by rotation.

The type of rotational-vibrational spectrum that will be observed can be determined qualitatively from a consideration of the configuration of the molecule. Thus, if in a linear molecule the vibration will cause a displacement of the dipole moment along the longitudinal axis, the spectrum will contain only the P- and R-branches. If the vibration causes a change in the dipole moment perpendicular to that axis, then the P-, Q-, and R-branches will be observed. Spherical top molecules will have the same type of rotational-vibrational spectrum as that observed for linear molecules with a dipole change perpendicular to the longitudinal axis.

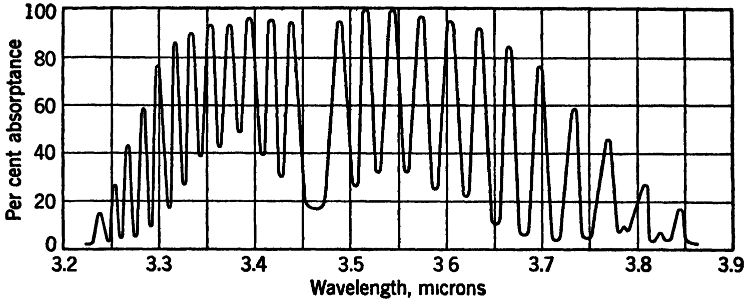

FIG. 8·1. The HCl band at 3.46 μ mapped with 7500-line grating. The HCl is at atmospheric pressure. [Imes, *Astrophys. J.*, **50**, 251 (1919).]

The vibrational-rotational spectra of asymmetric top molecules are complex, consisting in general of sets of sub-bands each of which has its own P-, Q-, and R-branches. In spite of this complexity, however, it is possible to classify these bands in three types, designated as A, B, and C, depending upon whether the change in dipole moment during the vibration is, respectively, along the least, intermediate, or greatest moment of inertia.

Not only can the type of rotational-vibrational spectrum that will be observed be determined from a consideration of the configuration of the molecule, but also the converse is true. That is, from a consideration of the type of rotational-vibrational spectrum observed the configuration of the molecule can be deduced. The information that can be derived from a study of the vibration-rotation spectrum is then (1) the moment of inertia of the molecule about the axis of rotation, giving the molecular dimensions, (2) the bond strength which is deduced from the fundamental frequency ν_0 and the mass of the molecule, and (3) the configuration of the molecule.

8·3 Sources. In common with other types of absorption spectrometers, infrared instruments require a source of radiant energy, a means of isolating narrow frequency bands, and a means for precisely measuring the energy transmitted by the absorbent. However, the source, the optical characteristics of the instruments, and the devices used to measure radiant energy in infrared spectrometers differ from those used in the visible and ultraviolet portions of the spectrum.

For the very near infrared, up to about 2 microns, the ordinary incandescent lamp serves very satisfactorily, but as a source for radiant energy in the far infrared it fails because of being glass enclosed and from having a low spectral emissivity. For most work, either the Nernst glower or the Globar is used. In addition, graphite rods heated electrically in an inert atmosphere or the Welsbach mantle may be used.

The Nernst glower is composed principally of rare earth oxides, such as zirconia, yttria, and thoria. The elements are usually shaped into short rods about 20 mm. long and 1 mm. in diameter. The ends are cemented to short ceramic tubes to facilitate mounting the glower, and the manufacturer fastens short platinum leads to the element for electrical connections. This source is nonconducting at room temperature and must be heated by external means to bring it to a conducting state. Like all semiconductors, it has a negative coefficient of resistivity, and, as a consequence, it must be operated in series with a ballast resistance in a constant-voltage circuit. Nernst glowers are usually operated in water-jacketed housings because of the heat emitted—about 100 watts. The emissivity of this source is low in the far infrared, but it has been used for wavelengths up to 25 microns. One serious objection to the Nernst glower is the frequent mechanical failure of the source due to the poor bonding of the platinum leads to the element itself, which necessitates frequent replacement of the glowers. Another objection is that its energy is largely concentrated in the visible and near infrared regions of the spectrum, making it necessary to filter out this objectionable radiant energy.

The Globar is extensively used as a source of infrared radiant energy. Except at the longest wavelengths, the energy from this source closely approximates that of a black body at the same temperature. This is shown in Fig. 8·2.

For spectrometric measurements beyond 20 microns, the Globar, having high emissivity in the far infrared, makes a good source. It is especially good when used in conjunction with a paraffin filter to make its effective total emissivity small. Short Globars, 5 cm. long and about 0.5 cm. in diameter, which have silvered ends to insure good electrical contact are on the market. They use approximately 200 watts at 40 to 50 volts and, like the Nernst glowers, operate within water-jacketed housings. Since the resistance of a Globar increases with the length of time used, provision must be made for increasing the voltage across it. This is usually accomplished by making a variable type transformer an integral part of the power supply. One objectionable characteristic of the Globar as a source is its relatively short life; this is true particularly if the operating temperature is allowed to increase substantially over 1200°C.

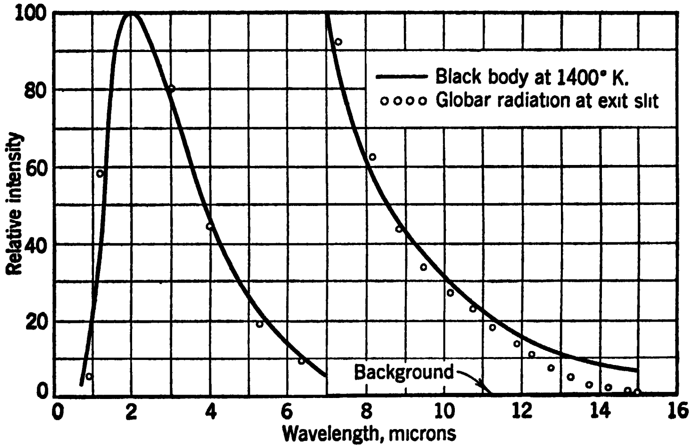

FIG. 8·2. Radiant energy curve for a black body at 1400°K. and for a Globar source. [McAlister, Matheson, and Sweeney, *Rev. Sci. Instruments*, **12**, 319 (1941).]

8·4 Spectrometers. The spectrometers used in the infrared portion of the spectrum belong to three main classes, distinguished by the means of dispersion which are utilized—prism, grating, or combination prism-grating instruments. Although there is some allowable flexibility in the manner in which the optical parts may be arranged, nearly all instruments are of the Littrow or the Wadsworth-Littrow types, in which the radiant energy traverses the prism twice, thus doubling the dispersion and the resolution ordinarily obtained.

Energy is very much at a premium throughout the infrared spectrum, particularly as the wavelength increases, owing to the rapidly diminishing intensity of all sources. For this reason, the prism spectrometer has a very distinct advantage over the grating, since it concentrates the dispersed energy into a single spectrum.

8·5 Prism materials. The limited range of transmission of most substances in the infrared limits seriously the choice of prism materials. Glass prisms can be used only to wavelengths of about 2.5 μ. Beyond this point prisms made from single crystals of the alkali halides, or quartz, are used, each for a limited region.

The ideal prism material should (1) be transparent over a wide wavelength range; (2) be available in large pieces of optical quality material; (3) be isotropic; (4) be resistant to moisture, fumes, and chemicals; and (5) have a high angular dispersion ($d\phi/d\lambda$), with a relatively small index of refraction-temperature coefficient (dn/dt). In addition, the prism material should be capable of taking a high polish. Optical materials satisfying all these requirements are not available, so compromises must be made.

The regions of transmission of the crystalline materials used as prisms in infrared spectroscopy are shown in Table 8·1.

TABLE 8·1 TRANSMISSION LIMITS OF PRISM MATERIALS USED IN INFRARED SPECTROSCOPY

Material	*Transmission Range*
Crystalline quartz	0.185 to 3.5 μ
Lithium fluoride	0.12 to 6.5
Fluorite	0.12 to 9
Rock salt	0.20 to 16
Sylvite	0.20 to 21 *
Potassium bromide	0.21 to 28
Potassium iodide	0.25 to 31

* Except for narrow absorption bands at 3.2 and 7.1 μ.

Where a selection of materials is possible, it is usually best to choose the substance which has its long wavelength transmission limit just above the region to be investigated. Since the dispersion increases rapidly as the transmission limits are approached, this choice will give the highest dispersion and the greatest resolving power. The most suitable prism materials for different portions of the infrared region are given in Table 8·2. Above 30 microns no material sufficiently transparent for prisms is known; therefore, gratings must be used as dispersing agents.*

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* Recently a thallium bromide iodide prism was used for measurements up to 38 microns.

TABLE 8·2 PRISM MATERIALS FOR DIFFERENT SPECTRAL REGIONS (*18*, *22*, *24*)

Region, Microns	*Material*
0.7 to 3.5	Quartz
3 to 6	Lithium fluoride
5 to 9	Fluorite
8 to 15	Rock salt
15 to 20	Sylvite
19 to 25	Potassium bromide

The angular dispersion $(d\phi/d\lambda)_D$, the refractive indices n_D, and the index of refraction-temperature coefficients $(dn/dt)_D$ for the alkali halides are given in Table 8·3.

TABLE 8·3 PHYSICAL PROPERTIES OF THE ALKALI HALIDES

Material	n_D	$(d\phi/d\lambda)_D$	$(dn/dt)_D$
Lithium fluoride	1.39177	0.286×10^{-5} *	-2.3×10^{-5} ‡
Fluorite	1.43385	0.333×10^{-5} †	-1.0×10^{-5} §
Rock salt	1.54431	0.938×10^{-5} †	-3.7×10^{-5}
Sylvite	1.49038	0.729×10^{-5} †	-3.6×10^{-5}
Potassium bromide	1.5581	1.449×10^{-5} *	-3.6×10^{-5} ‖
Potassium iodide	1.6634	2.881×10^{-5} *	-5.0×10^{-5} ‖
Crystalline quartz	1.54426	0.628×10^{-5} †	-0.5×10^{-5}

* Data from F. H. Perrin, *J. Optical Soc. Am.*, **28**, 86 (1938).

† *Dictionary of Applied Physics*, IV, 136.

‡ H. W. Hohls, *Ann. Phys.*, **29**, 433 (1937).

§ Ordinary ray.

‖ J. W. Forrest, *J. Optical Soc. Am.*, **32**, 382 (1942).

With the exception of potassium iodide, large crystals of these substances are grown artificially on a commercial basis. Lithium fluoride has been prepared to give 60° prisms with faces 10 × 15 cm. This material has a low index of refraction ($n_D = 1.39$), but its transmittance in the neighborhood of 2.7 microns is usually low, because of presence of impurities.

Fluorite crystals of a promising size have been artificially prepared. Since this material is more or less impervious to attack by water or ordinary fumes, future developments show promise.

Potassium iodide is very hygroscopic and is difficult to polish. Crystals of the other alkali halides are artificially grown to give 60° prisms with faces 10 × 15 cm. Such prisms are, however, quite readily attacked by water, and, as a result, great skill must be exercised in polishing their faces. Although they are not affected by atmospheric moisture under normal ranges of relative humidities, fluctuations in temperature may result in a deposition of moisture on the prism faces. For this reason, prisms of the alkali halides are kept in evacuated or desiccated chambers.

8·6 Dispersion. In considering the ideal dimensions for prisms, it is necessary to take into account the dispersion, the resolution, and the losses of radiant energy by reflection and absorption. In Fig. 8·3 is shown the path of a ray through a principal plane of a prism. The term *principal plane* is used to define a plane perpendicular to the plane formed by a refracting face of the prism. Since the angular deviation of the ray is ϕ, it can be seen that

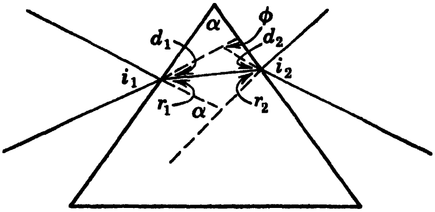

FIG. 8·3. Path of a ray through a principal plane of a prism.

$$\alpha = r_1 + r_2 \text{ and } \phi = d_1 + d_2 = i_1 - r_1 + i_2 - r_2 \tag{8·10}$$

$$= i_1 + i_2 - (r_1 + r_2)$$

$$= i_1 + i_2 - \alpha$$

At minimum deviation, $i_1 = i_2$ and $r_1 = r_2$, so that $\phi = 2i - \alpha$, and

$$i = \frac{\phi + \alpha}{2} \tag{8·11}$$

Also, since $\alpha = r_1 + r_2 = 2r_1$,

$$r = \frac{\alpha}{2} \tag{8·12}$$

Now, by Snell's law, $n = \sin i/\sin r$. Therefore, by substituting the values found for i and r_1,

$$n = \frac{\sin \dfrac{\phi + \alpha}{2}}{\sin \dfrac{\alpha}{2}} \tag{8·13}$$

This equation is of fundamental importance, since it is the basis of the method devised by Fraunhofer for the first accurate determination of the index of refraction.

By differentiation of equation (8·13),

$$\frac{dn}{d\phi} = \frac{\cos\dfrac{\phi + \alpha}{2}}{2\sin\dfrac{\alpha}{2}}$$

and

$$\frac{d\phi}{dn} = \frac{2\sin\dfrac{\alpha}{2}}{\cos\dfrac{\phi + \alpha}{2}} \tag{8·14}$$

Because $\cos(\phi + \alpha)/2 = \sqrt{1 - \sin^2(\phi + \alpha)/2}$, and, according to Snell's law, $n \sin \alpha/2 = \sin(\phi + \alpha)/2$, equation (8·14) becomes

$$\frac{d\phi}{dn} = \frac{2\sin\dfrac{\alpha}{2}}{\sqrt{1 - n^2 \sin^2\dfrac{\alpha}{2}}} \tag{8·15}$$

This gives us the value of $d\phi/dn$ in terms of the prism angle and the index of refraction. In practice, the simpler equation

$$\Delta\phi = (n_1 - n_2)\frac{2\sin\dfrac{\alpha}{2}}{\sqrt{1 - \bar{n}^2 \sin^2\dfrac{\alpha}{2}}} \tag{8·16}$$

is used where $\Delta n = n_1 - n_2$, and n, in equation (8·15), is replaced by the average

$$\bar{n} = \frac{n_1 + n_2}{2} \tag{8·17}$$

This equation is, of course, the more accurate the smaller Δn becomes, but the error amounts to only a few minutes when $\Delta\phi$ is as great as 9 degrees.

The angular dispersion $d\phi/d\lambda$ can be obtained by using the relationship

$$\frac{d\phi}{d\lambda} = \frac{d\phi}{dn} \cdot \frac{dn}{d\lambda} \tag{8·18}$$

For the evaluation of the ratio $dn/d\lambda$, Hartmann's (*27*) simple interpolation formula can be used over small ranges:

$$\lambda = \lambda_0 + \frac{C}{n - n_0}$$

and

$$n = n_0 + \frac{C}{\lambda - \lambda_0} \tag{8·19}$$

By differentiation,

$$\frac{dn}{d\lambda} = -\frac{C}{(\lambda - \lambda_0)^2} \tag{8·20}$$

so

$$\frac{d\phi}{d\lambda} = \frac{2 \sin \frac{\alpha}{2}}{\sqrt{1 - n^2 \sin^2 \frac{\alpha}{2}}} \frac{-C}{(\lambda - \lambda_0)^2} \tag{8·21}$$

To apply this equation, in finding the angular dispersion of a prism at any wavelength, λ, for which the index of refraction is n, the constants c and λ_0 must first be determined from equation (8·19).

Another equation, which gives n as a function of wavelength, is the one proposed by Cauchy:

$$n = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^4} + \cdots \tag{8·22}$$

where A, B, C, $\cdots$, are the characteristic constants of the prism material. The following equation is only approximate, but it will give results of sufficient accuracy for many purposes:

$$n = A + \frac{B}{\lambda^2} \tag{8·23}$$

By differentiation

$$\frac{dn}{d\lambda} = -\frac{2B}{\lambda^3} \tag{8·24}$$

This expression shows that the ratio $dn/d\lambda$ is inversely proportional to the cube of the wavelength.

The value of $d\phi/dn$ for a prism train can be expressed another way, one that is very convenient for certain purposes. Referring to equation (8·11), $(\phi + \alpha)/2 = i =$ angle of incidence, while equation (8·12) shows that $\alpha/2 = r =$ angle of refraction. Substituting in the expression for $d\phi/dn$, where

$$\frac{d\phi}{dn} = \frac{2 \sin \frac{\alpha}{2}}{\cos \frac{\phi + \alpha}{2}} \tag{8·14}$$

the following is obtained:

$$\frac{d\phi}{dn} = \frac{2 \sin r}{\cos i} = \frac{2 \frac{\sin i}{n}}{\cos i} = \frac{2}{n} \tan i \tag{8·25}$$

This gives the ratio $d\phi/dn$ as a function of the angle of incidence on the first prism face.

8·7 Resolving power of a prism. If the images of two adjacent spectral lines in the focal plane of a spectrograph are so close together that their central maxima overlap, the resultant intensity is obtained by summing up the intensities of the two individual maxima. It was proposed by the third Lord Rayleigh, as a criterion of resolving power, that two lines could be considered resolved if the central maximum of one line fell on the first minimum of the other. This case is portrayed in Fig. 8·4 for two lines of equal intensity. The resultant intensity obtained by summation of the two curves is shown by the dotted line.

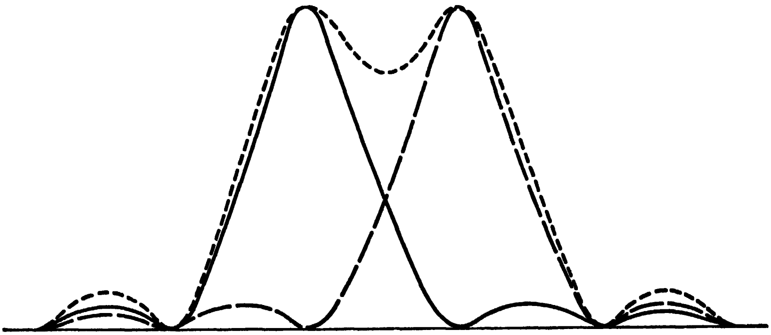

FIG. 8·4. Resulting intensity of two overlapping diffraction patterns (shown by dotted curve).

The resolving power of a spectrometer is expressed as the ratio of the mean wavelength of two rays that are just resolved to their wavelength difference. That is,

$$R = \frac{\bar{\lambda}}{\Delta\lambda} \tag{8·26}$$

The ratio $\bar{\lambda}/\Delta\lambda$ is, then, a pure number without dimensions which may vary widely for different instruments.

Lord Rayleigh showed that, in the case of the image of an infinitely narrow slit produced in the focal plane of a spectrograph, the linear distance ξ of the first diffraction minimum from the central maximum is given by the equation

$$a\xi = f\lambda \tag{8·27}$$

where a is the linear aperture of the collimator, and f is its focal length. Since ξ/f equals the angular distance between the central maximum and the first minimum, it is obvious that the angle, $\Delta\phi$, between two rays that are just resolved is expressed as

$$\Delta\phi = \frac{\bar{\lambda}}{a} \tag{8·28}$$

where $\bar{\lambda}$ is the mean wavelength of the rays.

Writing the resolving power, $\bar{\lambda}/\Delta\lambda$, in terms of the angular dispersion, the following expression is obtained:

$$R = \frac{\bar{\lambda}}{\Delta\lambda} = \frac{\bar{\lambda}}{\Delta\phi} \cdot \frac{\Delta\phi}{\Delta\lambda} = \frac{\bar{\lambda}}{\Delta\phi} \cdot \frac{d\phi}{d\lambda} \tag{8·29}$$

According to equation (8·28) $\bar{\lambda}/\Delta\phi = a$. When this value is substituted in equation (8·29), the resolving power is given as

$$R = \frac{\lambda}{\Delta\phi} \cdot \frac{d\phi}{d\lambda} = a\frac{d\phi}{d\lambda} \tag{8·30}$$

Since equation (8·18) shows that

$$\frac{d\phi}{d\lambda} = \frac{d\phi}{dn} \cdot \frac{dn}{d\lambda}$$

then

$$R = a\frac{d\phi}{dn} \cdot \frac{dn}{d\lambda} \tag{8·31}$$

Using the value of $d\phi/dn$ given in equation (8·15) as

$$\frac{d\phi}{dn} = \frac{2 \sin \frac{\alpha}{2}}{\sqrt{1 - n^2 \sin^2 \frac{\alpha}{2}}}$$

and remembering that $\sqrt{1 - n^2 \sin^2 (\alpha/2)} = \cos i$, the following value for the resolving power is obtained:

$$R = \frac{2a \sin \frac{\alpha}{2} \frac{dn}{d\lambda}}{\cos i} \tag{8·32}$$

Referring to Fig. 8·5, $a = l \cos i$, and $2l \sin (\alpha/2) = t$, so that

$$R = t \frac{dn}{d\lambda} \tag{8·33}$$

In this expression for resolving power it is assumed that the entire prism face is used. If such is not the case, the effective prism thickness, $(t - s)$, must be used in the above equation, where t and s are the thicknesses of prism material traversed by the extreme rays of the beam. Obviously, the resolving power of a train of prisms is equal to the algebraic sum of the resolving powers of the individual prisms. Since the resolving power depends on the effective thickness of the prism traversed, several small prisms will have no greater resolving power than one large one of the same effective thickness.

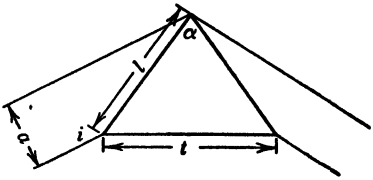

Fig. 8·5. Resolving power of a prism. Path shown is for a monochromatic beam.

8·8 Loss of energy by reflection. It must be remembered that the radiant energy refracted by the surface of a medium is partially polarized. Therefore, in dealing with the amount of energy reflected, it is necessary to apply the laws for polarized light. If i and r are the angles of incidence and reflection respectively, the fraction reflected at a surface of a light, plane polarized at right angles to the plane of incidence, is in accordance with the Fresnel equation:

$$X = \frac{\sin^2 (i - r)}{\sin^2 (i + r)} \tag{8·34}$$

The reflected fraction of light which is plane polarized in the plane of incidence is

$$Y = \frac{\tan^2 (i - r)}{\tan^2 (i + r)} \tag{8·35}$$

The transmitted fractions are, respectively, $1 - X$ and $1 - Y$. If ordinary light is regarded as composed of two beams of equal intensity polarized at right angles to one another, then the amount reflected will be $(X/2) + (Y/2)$, and the amount transmitted will be $\frac{1}{2}[(1 - X) + (1 - Y)]$. On meeting a second surface, the amount transmitted will be $\frac{1}{2}[(1 - X)^2 + (1 - Y)^2]$, and in general for m surfaces

$$T = \tfrac{1}{2}[(1 - X)^m + (1 - Y)^m] \tag{8·36}$$

For the case of normal incidence, $i = r = 0$, both Fresnel equations for the intensity of the reflected light take the form

$$X_0 = Y_0 = \frac{(n_1 - n_2)^2}{(n_1 + n_2)} \tag{8·37}$$

where n_1 and n_2 are the indices of the two media. If one medium is air,

$$X_0 = Y_0 = \frac{(n - 1)^2}{(n + 1)} \tag{8·38}$$

The fraction of light transmitted after reflection from m similar surfaces is

$$T_0 = (1 - X_0)^m \tag{8·39}$$

Except in cases of very divergent beams, this simple expression is adequate for calculating the transmittance of the surfaces of windows and cells.

8·9 Loss of energy by absorption. When radiant energy passes through a medium, a certain amount is absorbed. If I_0 is the intensity of the incident beam and I that of the transmitted beam, then by Bouguer's law

$$I = I_0 e^{-a_i b} \tag{8·40}$$

where a_i is the absorbance index, and b is the thickness of material. This expression may be written in the form

$$\log_e \frac{I_0}{I} = a_i b \quad \text{or} \quad \log_e \frac{1}{T_i} = a_i b \tag{8·41}$$

where T_i is the transmittance.

It is apparent that the logarithm of the reciprocal of the transmittance is proportional to the thickness of the medium traversed by the beam. In the case of prisms, b is equal to the average thickness, or half the base multiplied by the number of prisms. According to equations (8·31), (8·32), and (8·33), half the base of the prism $t/2$ is equal to

$$\frac{2a \sin \frac{\alpha}{2}}{\sqrt{1 - n^2 \sin^2 \frac{\alpha}{2}}} \tag{8·42}$$

But, from equation (8·15) the dispersion, $d\phi/dn$, is given as

$$\frac{d\phi}{dn} = \frac{2 \sin \frac{\alpha}{2}}{\sqrt{1 - n^2 \sin^2 \frac{\alpha}{2}}}$$

and for m identical prisms

$$m \frac{d\phi}{dn} = \frac{2m \sin \frac{\alpha}{2}}{\sqrt{1 - n^2 \sin^2 \frac{\alpha}{2}}} \tag{8·43}$$

Therefore, the logarithm of the amount of light absorbed, or the logarithm of the reciprocal of the transmittance, is proportional to the product of the dispersion and the aperture; that is,

$$\log_e \frac{1}{T_i} = a_i a m \frac{d\phi}{dn} \tag{8·44}$$

This is an important result, for it means that for spectrometers of equal dispersion, having prisms of the same material, the absorbance is the same in all. It makes no difference in the over-all absorbance whether a large number of prisms of small refracting angles or a smaller number with large angles are used, so long as both trains have the same dispersion and are made of the same material. The question of absorbance, therefore, does not enter into the question of the best dimensions for prisms.

8·10 Dimensions of prisms. In order to compare the relative values of prisms of different angles, it is desirable to compare prisms of equal resolving power. If the resolving power is kept constant while the refractive angle is changed, the following quantities will vary: the prism volume, the length of the refracting face, the aperture, the angle of incidence, the loss of light, and the dispersion, $d\phi/dn$. Wadsworth (*57*) calculated these quantities for different refracting angles, the length of the prism base being kept constant to maintain constant resolving power. Some of his results are shown in Table 8·4. Other values are reproduced in Baly (*5*), Vol. I, Chapter III.

TABLE 8·4 DISPERSION AND TRANSMISSION OF GLASS PRISMS OF EQUAL RESOLVING POWER WITH REFRACTIVE INDICES OF 1.5

Prism Angle	*Angle of Incidence*	*Aperture*	*Fraction of Light Transmitted*	*Dispersion,* $d\phi/dn$
30°	22° 51′	1.780	0.9207	0.5618
45°	35° 2′	1.070	0.9163	0.9348
55°	43° 45′	0.782	0.9047	1.278
60°	48° 35′	0.661	0.8953	1.512
68°	57° 1′	0.487	0.8588	2.054
75°	65° 57.5′	0.335	0.7720	2.988

Prisms of small angles transmit more light than prisms of larger angles and have, moreover, a larger aperture. They are, however, inferior in dispersion. For a given aperture it is best to choose prism angles near the angle affording maximum polarization. At minimum deviation this angle is 67° 22′ for $n = 1.5$, 64° 0′ for $n = 1.6$, and 60° 56′ for $n = 1.7$. Moreover, it must be remembered that it is much more difficult to make prisms of large angles than those of small ones because increased distortion in the spectrum is produced by imperfections in the refracting faces. All these considerations, then, favor 60° prisms for infrared spectroscopy.

From Fig. 8·5, it is seen that the aperture of a prism, a, is given by the equation

$$a = l \cos i$$

In order that the prism just accept the collimated beam, the height of the prism should be equal to $l \cos i$. Since

$$\cos i = \sqrt{1 - n^2 \sin^2 \frac{\alpha}{2}}$$

the height, h, is given as

$$h = l\sqrt{1 - n^2 \sin^2 \frac{\alpha}{2}} \qquad (8\cdot45)$$

and for 60° prisms,

$$h = l\sqrt{1 - \frac{n^2}{4}} \qquad (8\cdot46)$$

8·11 Curvature of lines in prism spectrometers. With the exception of double monochromator instruments of the zero-dispersion type, all prism type spectrometers with straight entrance slits form curved spectral lines at the exit slits. This is due to the fact that only rays from the center of the slits are rendered parallel by the collimators and pass through the principal planes of the prisms. Rays from slit points other than on the collimator axes must traverse the prisms obliquely, the angle becoming larger as the distance of the point source from the collimator axes is increased. Since these rays traverse greater prism thicknesses than do parallel rays, they are, in effect, dispersed by prisms of greater angles and are, consequently, deviated more. Hence, the images of the entrance slits are curved, with the convex sides turned toward the red end of the spectrum. The theory of this curvature is reviewed up to 1895 by Kayser (*32*).

To a first approximation, the spectral lines are parabolic in shape and the parabola lies in a plane which is normal to the central ray passing through the principal planes of the prism. The line in the plane containing the parabola which is parallel to the refracting edge of the prism may be taken as the y-axis, and the line in the plane containing the parabola which is perpendicular to the refracting edge of the prism may be taken as the x-axis. Then the equation defining the position of the spectral line is

$$x = \frac{n^2 - 1}{2nf} \frac{dD}{dn} y^2 \tag{8·47}$$

where n is the refractive index, f is the focal length of the collimator and D is the angular deviation (in radians) produced by the prism system. This equation holds for both minimum deviation and for the case in which the entrance angle is fixed, as in the spectrometer.

Now, the radius of curvature, ρ, of any curve is given as

$$\rho = \frac{\left[1 + \left(\frac{dy}{dx}\right)^2\right]^{3/2}}{\frac{d^2y}{dx^2}}$$

which for a parabola at the vertex becomes .

$$\rho = \frac{nf}{n^2 - 1} \frac{dn}{dD} \tag{8·48}$$

For a prism at minimum deviation, $D = 2i - \alpha = \phi$, so that $dn/dD = -dn/2di = dn/d\phi$. But the dispersion, $d\phi/dn$, according to equation (8·15), is equal to

$$\frac{d\phi}{dn} = \frac{2 \sin \frac{\alpha}{2}}{\left(1 - n^2 \sin^2 \frac{\alpha}{2}\right)^{1/2}} \tag{8·15}$$

so that

$$\rho = \frac{nf}{2} \frac{\left(1 - n^2 \sin^2 \frac{\alpha}{2}\right)^{1/2}}{(n^2 - 1) \sin \frac{\alpha}{2}} \tag{8·49}$$

An alternative expression is obtained by using the value of $d\phi/dn$ obtained in equation (8·25), as

$$\frac{d\phi}{dn} = \frac{2}{n} \tan i \tag{8·25}$$

which, when substituted in equation (8·48), yields for the radius of curvature

$$\rho = \frac{n^2 f}{2(n^2 - 1) \tan i} \tag{8·50}$$

In infrared spectroscopy it is usually desirable to have the spectral lines straight at the exit slit. This is done by curving the entrance slit, the radius of curvature being made equal to that of the vertex of a spectral line at the desired wavelength. Since the spectrum produced by a prism is curved with the convex side toward the refracting angle of the prism, the proper orientation of the slit can easily be found by tracing the path of the radiant energy through the spectrometer.

According to equation (8·33), the resolving power of a spectrometer is given as

$$R = t \frac{dn}{d\lambda}$$

Partially to compensate for the lower resolving power of the prism in the wavelength interval where $dn/d\lambda$ is at a minimum, the entrance slit is usually curved with a radius of curvature equal to that of the vertex of a spectral line from this region. For rock-salt prisms, $dn/d\lambda$ is at a minimum near 2.9 microns, where the refractive index is 1.5245.

8·12 The effect of temperature on prism spectrometers. It was noted in Section 8·5 that the temperature coefficient of the index of refraction ranges from -0.5×10^{-5} for crystalline quartz to -5×10^{-5} for potassium iodide. This means that changes in temperature may lead to serious difficulties in prism spectrometers through a shift in spectral positions. Since optical materials are all poor thermal conductors, considerable time is required for the whole prism to reach thermal equilibrium after a change in the surrounding temperature. Until equilibrium has been attained, the temperature gradients in the prism lead to variations in the index of refraction and consequent broadening of the spectral line. Merton (*38*) found that, if the prism is mounted in good thermal contact with the metal base supporting the prism and is covered, except for the refracting faces, with a close-fitting metal cover attached to the base, the gradients are made so uniform that they do not noticeably affect the definition.

Some instruments, especially small routine type spectrometers, have been equipped with mechanisms which automatically compensate for shifts in spectra positions due to changes in the temperature. These devices, which depend on bimetallic elements to alter the optical arrangement sufficiently to compensate for the spectral shift at a given wavelength, have proved to be very satisfactory. In general, the mechanisms rotate slightly either the Littrow mirror or the small flat mirror which directs the dispersed radiant energy onto the exit slit. The bimetallic elements should, obviously, be located as near the prism as possible.

Some spectroscopists prefer to standardize their spectrometers at frequent intervals in order that corrections for shifts in spectral positions can be applied when necessary. Since the last traces of water vapor in a spectrometer are removed only with the greatest difficulty, an absorption band due to water vapor is frequently used as the reference wavelength. Other reference lines commonly used are the emission lines of either the sodium- or mercury-arc lamps.

8·13 Gratings. Where higher dispersion and resolution are required, as for the study of the fine structure of bands in the near infrared, or for the accurate measurement of wavelengths, grating spectrometers are used. The gratings are usually of the type called by Wood "echelette gratings." The grooves are V-shaped with plane surfaces and are deep enough so that no flat surface remains between successive grooves. The shape of the groove and its inclination to the original surface may be selected at will and are made so as to concentrate the desired wavelength as far as possible into a single order.

Unfortunately, the resolution of grating instruments in the region from 8 to 25 microns is no greater than that given by large, well-designed prism spectrometers. This is due to the fact that the theoretical resolving power of gratings used for infrared work is seldom realized (*51*). The limit is set, not by the grating, but by the slit widths required for adequate illumination. The present limit of resolving power is 0.25 cm.^{-1} in the region from 1.5 to 7 microns and 0.4 cm.^{-1} at greater wavelengths (*54*).

8·14 Dispersion of a grating.* A diffraction grating is made up of an array of grooves, parallel, equidistant, and close together. A section of a plane reflection grating is shown diagrammatically in Fig. 8·6.

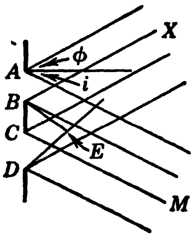

FIG. 8·6. Schematic diagram of a reflection grating.

In Fig. 8·6 a parallel beam of light falls obliquely on the grating from M, making an angle of incidence i with the normal. A large portion of the light passes into the apertures, but a portion is diffracted in the direction of X, the diffracted rays making an angle of diffraction ϕ with the normal. If now the perpendiculars DE and BE are drawn, it is seen that the difference in the length of the path traversed by the corresponding rays in each pencil, the so-called retardation, is given by the difference of the lengths BE and DE. Because the ray MB is retarded on the corresponding ray MD by an amount equal to the length BE, while the ray DX is retarded on the ray BX by an amount equal to DE, the total retardation is equal to $BE - DE$. Since

$$BE = d \sin i$$

and

$$DE = d \sin \phi \tag{8·51}$$

the total retardation equals $d(\sin i - \sin \phi)$, where d is the grating constant.

When this value is equal to one or more whole wavelengths, no interference takes place, so a bright image is seen. In general, then,

$$m\lambda = d(\sin i \pm \sin \phi) \tag{8·52}$$

where m is the order of the spectrum and λ is the wavelength. The retardations are to be added when both the incident and the diffracted rays are on the same side of the normal.

* For a detailed discussion of diffraction gratings see:

E. C. C. Baly, *Spectroscopy*, Vol. I, Chaps. VI and VII, Longmans, Green, London, 1924.

R. W. Wood, *Physical Optics*, 3rd ed., Chap. 7.

H. A. Rowland, *Phil. Mag.* [5], **35**, 397 (1893); *Astronomy and Astrophysics*, **12**, 129 (1893).

D. F. Meyer, *The Diffraction of Light, X-Rays, and Material Particles*, Chap. VI, The University of Chicago Press, Chicago, 1934.

R. A. Sawyer, *Experimental Spectroscopy*, Chap. 6, Prentice-Hall, New York, 1944.

R. F. Stamm and J. J. Whalen, *J. Optical Soc. Am.*, **36**, 2 (1946).

G. R. Harrison, R. C. Lord, and J. R. Loofbourow, *Practical Spectroscopy*, Prentice-Hall, New York, 1948.

If the angle of incidence is assumed to be constant, then by differentiation,

$$m\,d\lambda = d\cos\phi\,d\phi$$

and

$$\frac{d\phi}{d\lambda} = \frac{m}{d\cos\phi} \tag{8·53}$$

Thus, the dispersion of a grating is equal to the number of the order of the spectrum divided by the product of the grating space and the cosine of the angle of diffraction. It is evident that the dispersion reaches a minimum when ϕ equals zero.

A characteristic feature of gratings is the overlapping of spectra of different orders. Equation (8·52) shows that, for a fixed value of i and ϕ, if a wavelength λ is observed in the first order where $m = 1$, there will also appear wavelengths of higher-order spectra such that $\lambda_1 = 2\lambda_2 = m\lambda_m$. It is not often, in infrared work, that the overlapping orders can be separated by filters. For that reason fore-prisms are usually used for that purpose.

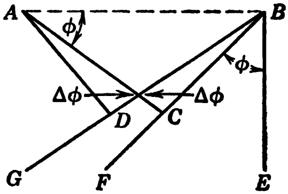

FIG. 8·7. Diffraction by a reflection grating.

8·15 Resolving power of a grating. In Section 8·7 the resolving power was expressed as the ratio of the mean wavelength of two rays that are just resolved to their wavelength difference; that is, according to equation (8·29), $R = (\bar{\lambda}/\Delta\phi)\cdot(d\phi/d\lambda)$. From this expression the resolving power of a grating is easily determined.

In Fig. 8·7, AB represents a grating, and AC and AD, the wave fronts of two rays just resolved of wavelengths λ and $\lambda + \Delta\lambda$, respectively. By equation (8·53)

$$\frac{d\phi}{d\lambda} = \frac{m}{d\cos\phi}$$

so

$$R = \frac{\bar{\lambda}}{\Delta\phi}\cdot\frac{d\phi}{d\lambda} = \frac{\bar{\lambda}}{\Delta\phi}\cdot\frac{m}{d\cos\phi} \tag{8·54}$$

But, according to equation (8·28), $\Delta\phi = \lambda/a$.

The aperture of the grating, a, is given as

$$a = AB\cos\phi = Nd\cos\phi$$

where d equals the grating space and N their number. It follows that the resolving power of a grating depends only on the product of the order and the number of rulings in the grating. That is,

$$R = a \cdot \frac{m}{d \cos \phi} = Nd \cos \phi \cdot \frac{m}{d \cos \phi} = mN \qquad (8\cdot55)$$

To resolve the sodium D lines in the first order, the required resolving power is $\bar{\lambda}/\Delta\lambda = 5893/5.967 = 987$; therefore, a grating with 987 ruled lines will have this theoretical resolving power. Very large theoretical resolving powers are, in fact, very nearly attained in practice throughout the visible and ultraviolet portions of the spectrum, but in the infrared are seldom realized (*51*).

If both sides of equation (8·52) are multiplied by N, the number of rulings in the grating, the following expression is obtained:

$$mN\lambda = Nd(\sin i \pm \sin \phi) \qquad (8\cdot56)$$

When the value of R in equation (8·55) is substituted in this expression, a valuable relation results:

$$R = \frac{Nd}{\lambda} (\sin i \pm \sin \phi) \qquad (8\cdot57)$$

Because the product, Nd, equals the width of the grating, this expression for the resolving power depends directly only on the aperture and the position into which the grating is turned and inversely on the wavelength. The maximum value that R can have is obtained when $i = \phi = 90°$. This maximum, however, cannot be attained in practice, as with very large values of the angles of incidence and diffraction the aperture becomes vanishingly small, and the radiant energy diffracted reaches a minimum. With Littrow type instruments, when $i = \phi$, the largest practical values the angles can have lie between 50° and 60°, while the resulting resolving power is somewhere between $0.95(Nd/\lambda)$ and $1.65(Nd/\lambda)$.

8·16 Infrared detectors and amplifiers. Photographic and photoelectric methods can be used to detect radiant energy in the near infrared up to about 2 microns. Beyond that there is no alternative to the thermocouple (*8*, *12*, *15*, *20*, *30*, *34*, *39*, *41*, *44*, *45*, *47*, *53*) or bolometer (*33*, *35*). The thermocouples usually employed are of the low-resistance type and normally have two independent junctions and receivers. This allows the thermocouple to be made compensating by connecting the junctions in opposition. Ordinarily, the radiant energy to be measured is focused on one receiver; the compensating junction acting as an external resistance in the detecting circuit. Although the response is somewhat diminished by this arrangement, the reduction of first- and second-order drifts justifies compensation and the attendant smaller response. The bolometers may be of either the metallic or nonmetallic thermistor types. Such detectors may or may not use compensating receivers. Those which employ no compensating receivers depend on other means to eliminate drift.

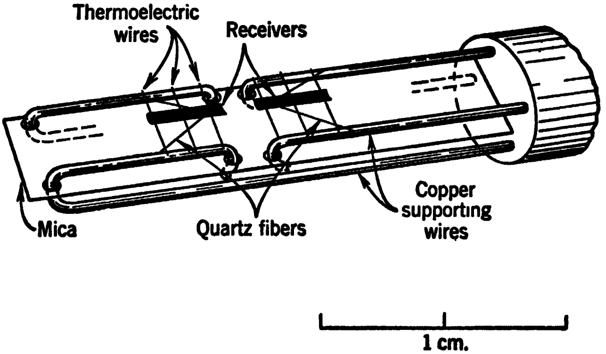

FIG. 8·8. Construction details of a thermocouple.

Figure 8·8 shows the details of the construction of a thermocouple. Quartz fibers are used to support the blackened gold foil receiver to which is fastened the junctions. One thermoactive wire is made of pure bismuth, and the other is an alloy of bismuth and 5 per cent tin. The selection of this combination of materials was made after a consideration of the Wiedemann-Franz coefficients, as well as the thermoelectric powers of various possible combinations, including such metals as tellurium and other bismuth alloys (*14*). The complete thermocouple and housing are shown in Fig. 8·9.

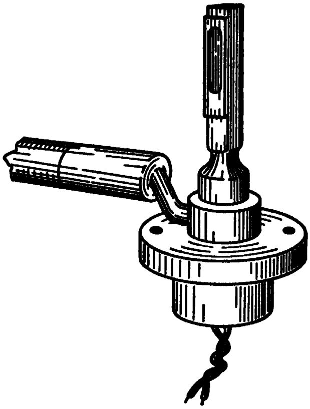

FIG. 8·9. A complete thermocouple.

When designing a thermocouple, the following considerations must be made. At equilibrium the rate at which radiant energy incident on a receiver is absorbed, Φ_a, is balanced by the rate at which energy is lost through conduction, reradiation, and the Peltier effect. That is,

$$\Phi_a = L_1\,\Delta T + L_2\,\Delta T + L_3\,\Delta T + L_4\,\Delta T \qquad (8\cdot58)$$

where L_1 represents the heat loss through reradiation from the receiver, L_2 the loss by air conduction, L_3 the loss through conduction of the wires and fibers, and L_4 the Peltier heat loss. ΔT is the temperature difference between the receiver and its surroundings. Ordinarily the Peltier loss may be neglected.

If A equals the area of the receiver, k the total thermal conductivity of the thermoelectric wires, quartz fibers, etc., σ the Stefan-Boltzmann radiation constant, T the absolute temperature of the receiver, e its effective radiating power, and ΔT the temperature difference between the receiver and its surroundings as a result of the radiant energy incident on it, then

$$\Phi_a = 4\sigma eAT^3\,\Delta T + k\,\Delta T \tag{8·59}$$

The term $4\sigma eAT^3\,\Delta T$ represents the heat loss by radiation and gas conduction. Where the receiver is not in a high vacuum, gas conduction has the same effect on thermocouple design as increasing the magnitude of e.

Since

$$\Delta T = \frac{\Phi_a}{4\sigma eAT^3 + k} \tag{8·60}$$

it is evident that, as the area of the receiver increases, the sensitivity decreases, the decrease becoming finally almost a linear function of A. These considerations, then, favor a thermocouple with very small receivers. Other considerations in spectrometer design, however, limit the reduction in size of the image of the exit slit to not more than seven fold. As a result, the area of the receiver is normally one-fourth to one-seventh the area of the exit slit when it is fully opened.

If E is the electromotive force and P is the thermoelectric coefficient, it is evident that

$$E = P\,\Delta T \tag{8·61}$$

and

$$E = \Phi_a \frac{P}{4\sigma eT^3A + k} \tag{8·62}$$

Moreover,

$$I = \Phi_a \frac{P}{\left(4\sigma eT^3A + \frac{a_1}{l_1}k_1 + \frac{a_2}{l_2}k_2\right)(R_t + R_e + R_g)} \tag{8·63}$$

where R_t equals the thermocouple resistance, R_g represents the galvanometer resistance, and R_e is any other resistance in the galvanometer-thermocouple circuit. In this expression a_1 and a_2 are the cross-sectional areas of the two thermoelectric wires, l_1 and l_2 are their lengths, while k_1 and k_2 are specific conductivity constants. R_1 and R_2 represent the resistance of the two thermocouple wires. Since

$$R_1 = \frac{l_1}{a_1}\rho_1 \tag{8·64}$$

where ρ is the specific electrical resistivity,

$$\frac{a_1}{l_1}k_1 = k_1\frac{\rho_1}{R_1} \tag{8·65}$$

Expressing equation (8·63) as a function of A, R_1, and R_2, the following value is obtained:

$$I = \Phi_a \frac{P}{\left(4\sigma eT^3A + \dfrac{k_1\rho_1}{R_1} + \dfrac{k_2\rho_2}{R_2}\right)(R_1 + R_2 + R_e + R_g)} \tag{8·66}$$

With R_g and R_e fixed, I reaches a maximum when

$$R_1 + R_2 = R_g + R_e \tag{8·67}$$

and

$$4\sigma eT^3A = \frac{k_1\rho_1}{R_1} + \frac{k_2\rho_2}{R_2} \tag{8·68}$$

In choosing a galvanometer for use with a thermocouple it should be noted that in general the voltage sensitivity of galvanometers changes little with R_g, but that as R_g increases the current sensitivity also increases.*

It is often desirable to amplify the galvanometer deflections so that small deflections become easier to measure and so that a continuous record can be made of the radiant energy reaching the thermocouple. A convenient means of doing this is to use an optical amplifier.

The Moll and Burger thermorelay may be used for amplifying galvanometer deflection until Brownian motion of the galvanometer becomes troublesome (*40*).

Other amplifiers include the barrier-layer photocell amplifier described by Barnes and Matossi (*7*) and the thermocouple with two triangular-shaped receivers described by Cartwright (*13*).

In Fig. 8·10 is portrayed the Barnes-Matossi amplifier. To make such an amplifier, the sensitive disc of a Weston Photronic cell is removed from the case and the conducting layer on the top surface is divided into two parts. This may be done by scratching a line along a diameter of the disc with a sharp instrument. Any loose particles must be removed. As a result of this operation, if properly carried out, the two halves of the top conducting layer will be insulated from each other.

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* For further discussion of thermocouple design and construction refer to John Strong, *Procedures in Experimental Physics*, Chap. 8, Prentice-Hall, New York, 1942.

The optical arrangement of the system is simple. An automobile headlight bulb is focused on the mirror of the primary galvanometer by a simple lens in the plane of which is a rectangular slot. The galvanometer lens forms an image of the slot on the divided disc of the photovoltaic cell. The two halves of the top conducting layer are connected to the secondary galvanometer, which indicates the difference in the illumination on the two sides of the center line. Such an arrangement permits current amplification of 200 to be obtained easily. This is sufficient to make Brownian motion quite noticeable. Unfortunately the linearity of response is questionable, and as a result frequent checks must be made. Such an amplifier also amplifies the drift of the primary galvanometer. This is undesirable.

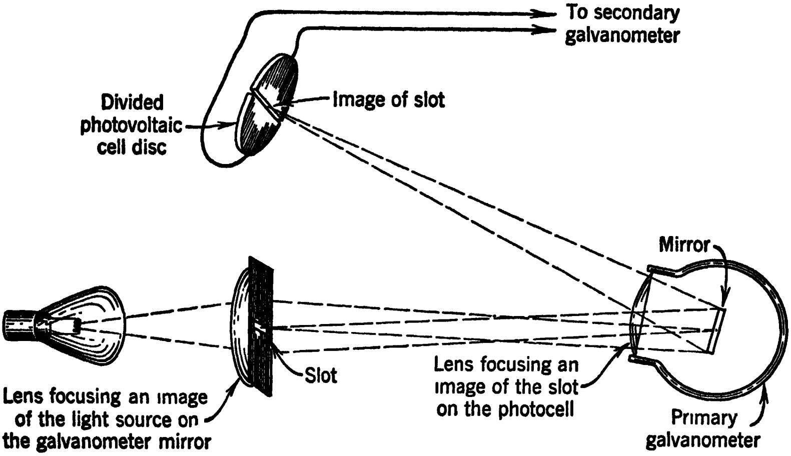
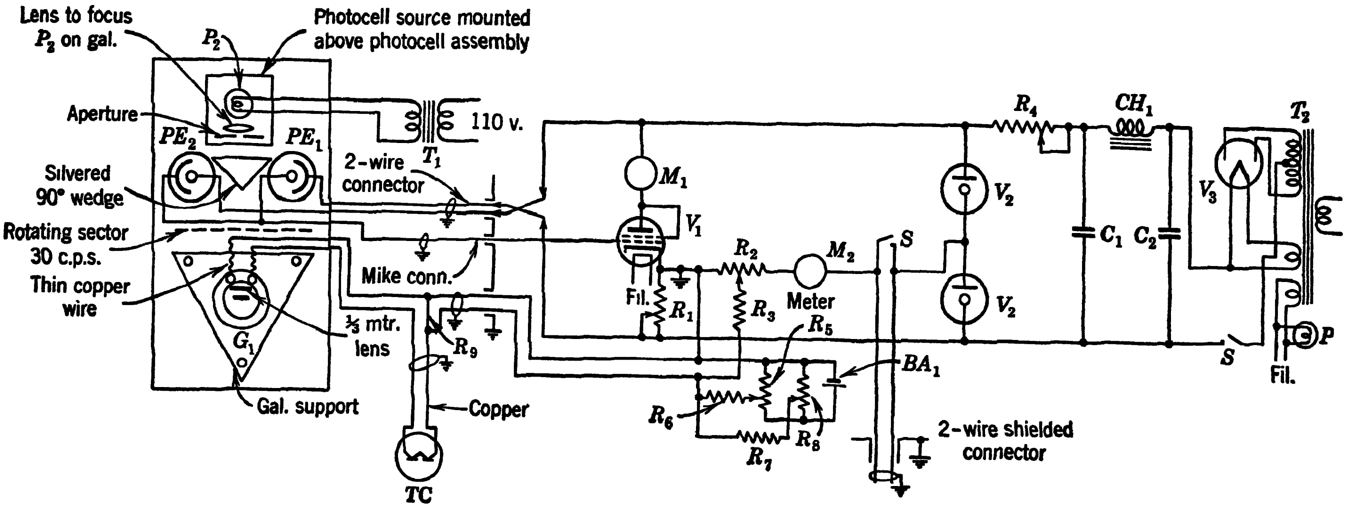

To secondary galvanometer
Divided photovoltaic cell disc
Image of slot
Mirror
Slot
Lens focusing an image of the slot on the photocell
Lens focusing an image of the light source on the galvanometer mirror
Primary galvanometer

FIG. 8·10. Barnes-Matossi amplifier. (Courtesy of R. B. Barnes.)

Pfund (*49*) and Hardy (*26*) devised a resonance amplifier which tends to eliminate drift of the galvanometer and to make the amplifier respond only to the radiant energy incident on the thermocouple. Their scheme is rather elaborate and not readily adapted to industrial use. It requires two identical galvanometers and a tuned pendulum shutter. The system is adjusted so that both the primary and secondary galvanometers are underdamped and adjusted to the same period. Then, by interrupting the radiant energy falling on the thermocouple with a periodicity corresponding to that of the galvanometers, a condi-



Code	Part	Code	Part	Code	Part
V_1	Type 38 vacuum tube	R_6	500-ohm fixed resistor—1 watt	M_1	0–25 ma.
V_2	Type VR105 voltage regulator tubes	R_7	5000-ohm fixed resistor—1 watt	M_2	0–10 ma.
V_3	Type 523 rectifier	R_8	1000-ohm potentiometer	$PE1$	Photocell—CE2A
C_1	16-mfd. condenser, electrolytic	R_9	15-cm. 18-gage copper wire	$PE2$	Photocell—CE2A
C_2	1-mfd. condenser, electrolytic or paper	BA_1	1.5-volt instrument battery	TC	Thermocouples in spectrometer
R_1	10,000-ohm potentiometer—10 watts	T_1	6 volt, 8 amp. sec.	G_1	Galvanometer type H.S.—L & N, 0.05 μv./mm. at 1 meter
R_2	500-ohm potentiometer	T_2	Plate and filament transformer; 350-0-350 60 ma. sec.; 5 volts, 3 amp.; 6.3 volts, 3 amp. sec.	P_1	Panel pilot
R_3	10,000-ohm fixed resistor	CH_1	Choke—30 henries, 60 ma.	P_2	32 cp. headlight bulb
R_4	5000-ohm adjustable resistor—50 watts				
R_5	1000-ohm potentiometer				

FIG. 8·11. Photoelectric amplifier.

tion of resonance is set up. As a result random disturbances are eliminated, but unfortunately this does not include lack of response to Brownian motion of the primary galvanometer.

Firestone (*21*) made an ingenious variation of the Pfund scheme. A photocell amplifier is used to charge and discharge a condenser through the secondary galvanometer. Since the secondary galvanometer circuit has infinite ohmic resistance, owing to the condenser in the circuit, no net current can flow, and as a consequence all deflections are excursions about an unchanging zero position.

McAlister, Matheson, and Sweeney (*37*) described an amplifier which has been widely accepted. This instrument, which was constructed along the lines of Weston's photoelectric potentiometer, is shown diagrammatically in Fig. 8·11.

The amplifier operates on 110 volts alternating current stabilized with a voltage regulator. In addition a 1.5-volt instrument battery is used. In series with the thermocouple *TC* is the galvanometer G_1 and a resistor R_g. R_g is a 15-cm. length of 18-gage copper wire. The galvanometer is a Leeds and Northrup type H.S. with a sensitivity of 0.05 microvolt/mm. at 1 meter and an internal resistance equal to that of the thermocouple.

A beam of light from the galvanometer mirror is divided at the apex of a reflecting prism, the two portions falling on gas-filled photocells which control the grid of the type 38 amplifier tube. The circuit is arranged so that a portion of the amplified current may be fed back through the resistor R_g. The amplifier gain which is determined by the amount of feedback is regulated by the potentiometer R_2. Potentiometers R_5 and R_8 control the zero of the amplifier.

The equipment behaves essentially as a null point instrument, since its action is such that, when an e.m.f. is developed in the thermocouple, the galvanometer swings and unbalances the photocell circuit. This causes the amplifier to feed an opposing current back into the galvanometer circuit through resistance R_g and almost restores the original balance. The feedback characteristic reduces the over-all period for recording a change in e.m.f. from the thermocouple and at the same time minimizes the zero drift due to room-temperature variations. Brownian motion of the galvanometer is reduced.

As originally designed, no provision was made for interrupting the light beam from the galvanometer to the photocells. It was found by the author, however, that, when a fixed beam of light struck the reflecting prism and the output of the amplifier was recorded, the record showed slow erratic excursions about a mean value. By interrupting the beam of light at the rate of 30 to 60 c.p.s. such erratic behavior was eliminated. A sector rotated by a small synchronous motor was used to interrupt the light beam.

The disadvantages of galvanometer type amplifiers are the slowness of response and the sensitivity to vibrations which make them unsuited to many applications. Liston, Quinn, Sargent, and Scott (*36*) described an electronic amplifier which has replaced to a large extent the galvanometer type amplifier. The instrument is as sensitive as the galvanometer and moreover is compact and simple to operate; most important, it is free from the difficulties caused by vibration. The drift is no greater than that associated with the McAlister-Matheson-Sweeney amplifier and the signal-to-noise ratio approaches closely the theoretical limit imposed by thermal agitation, the so-called Johnson noise, in the input circuit.

A schematic block diagram of this breaker type amplifier is shown in Fig. 8·12.

The high amplification associated with the instrument is obtained by converting the d-c. voltage across the input into an a-c. voltage by means of a single-pole, double-throw motor-driven breaker switch operating at about 75 c.p.s. This frequency was chosen to minimize the effects of induced signals from 60-cycle sources. To make the noise as low as possible there is a short period of overlap in the breaker cycle when both contacts are closed. The breaker applies the voltage developed by the thermocouple alternately to each half of the primary of a set-up transformer. The a-c. voltage from the secondary of this transformer is amplified by a conventional type three-tube resistance-capacity coupled amplifier. This is tuned to give maximum response at the breaker frequency. The a-c. voltage from the output transformer is rectified by an output breaker whose operation is synchronized with the breaker in the input circuit. This arrangement tends to reduce the effects of induced voltages and tube noise. A complete wiring diagram of the amplifier is given in Fig. 8·13, and in Fig. 8·14 is shown the front view of the amplifier.

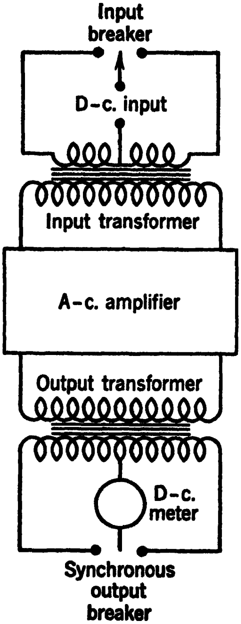


FIG. 8·12. Schematic block diagram of breaker type amplifier. (Courtesy of the Perkin-Elmer Corporation.)

Since the instrument was designed to operate with a 5- to 20-ohm input impedance, it is ideally suited for use with low-resistance thermocouples. It may be used with input impedances considerably beyond

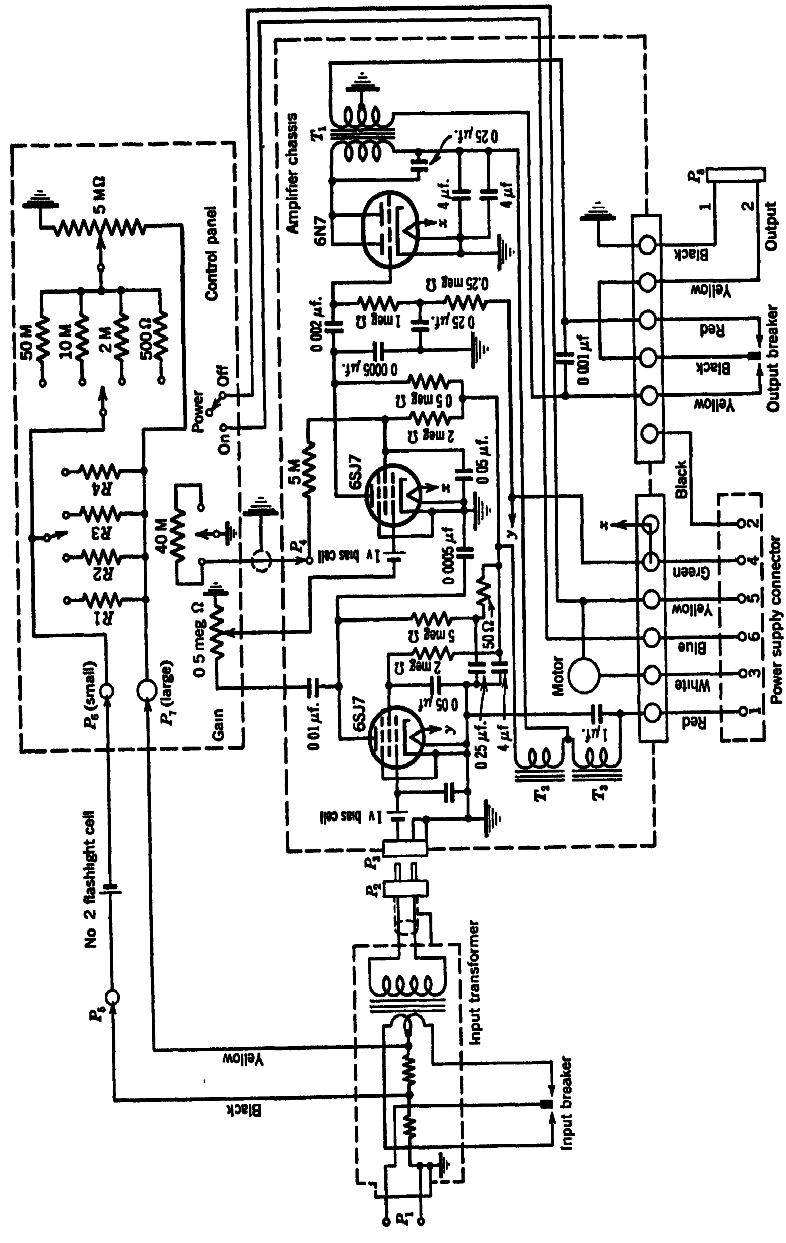


FIG. 8·13. Breaker type amplifier circuit diagram. (Courtesy of the Perkin-Elmer Corporation.)

the 20-ohm range but with a decrease in relative gain. At maximum gain, the amplifier is capable of supplying 2 milliamperes into a 500-ohm load when a 0.1-microvolt signal is sent into 10-ohm input circuit. Figure 8·15 shows that with a 500-ohm output load its response is linear for input voltages between 0.1 and 0.4 microvolt. By sacrificing gain the departure from linearity can be reduced a very considerable extent.

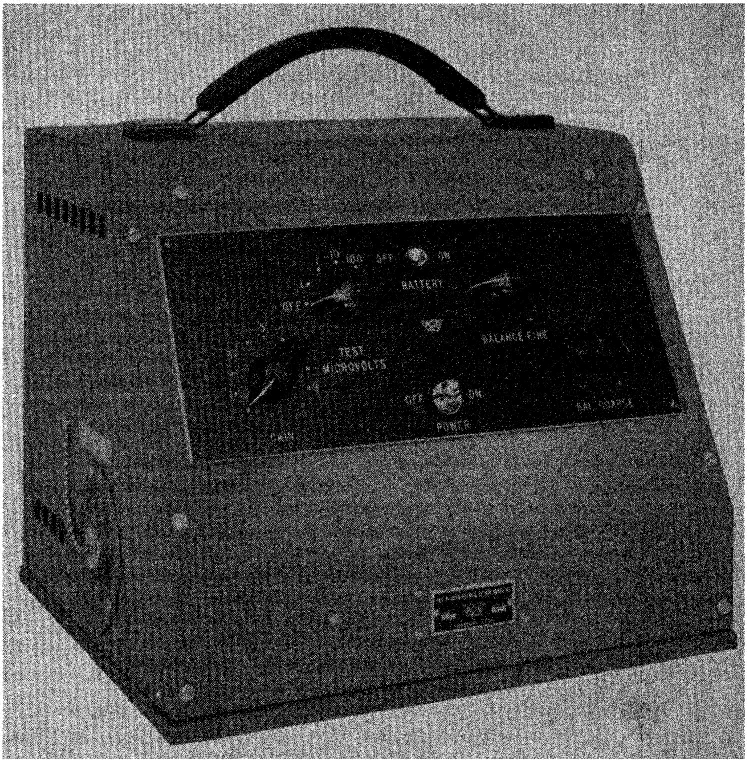


FIG. 8·14. Front view of breaker type amplifier. (Courtesy of the Perkin-Elmer Corporation.)

Other amplifiers which use no galvanometers have been developed to amplify the output of thermocouples. The wiring diagram of one described by Roess (*52*) is shown in Fig. 8·16.

The high amplification associated with this instrument is obtained by applying the alternating output of a rapid-response thermocouple to the primary of a step-up transformer. The alternating response from the thermocouple is obtained by interrupting the radiant energy incident on the thermocouple at frequencies between 1 and 5 c.p.s.

The a-c. voltage from the secondary of the transformer is further amplified with a resistance-capacity coupled amplifier connected in push pull. Twin-T resistance-capacity networks connected to provide negative feedback from the plates to the grids of one stage are used to reduce the noise in the amplifier output. The output of the amplifier is connected through a blocking condenser to a galvanometer. The entire swing of the galvanometer is recorded photographically, so that the resulting records have the appearance of modulated carrier waves similar to those first published by Firestone (*20*).

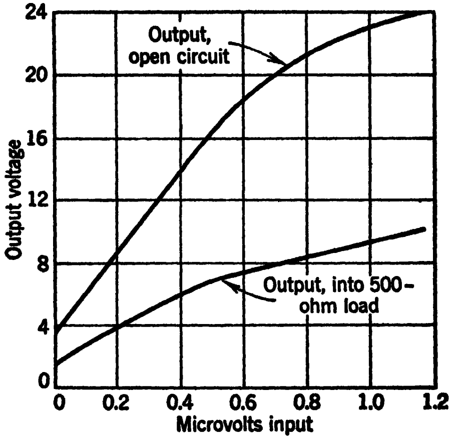


Fig. 8·15. Gain and output characteristics of 53-series breaker type amplifier. (Courtesy of the Perkin-Elmer Corporation.)

The amplifier is overelaborate in design and suffers from the fact that it uses photographic methods to make permanent records. It has much to recommend it, however. The modulated light beam incident on the thermocouple receiver should eliminate all drift. The response is linear, and the gain of the amplifier is limited only by the Johnson noise in the thermocouple itself.

The bolometer was devised by Langley (*33*) in 1880. It consists of two nearly identical, very thin strips of metal which form two arms of a Wheatstone bridge. The strips are blackened on one side to increase their ability to absorb radiant energy. One strip is hidden, and the other is exposed to the radiant energy which it is desired to measure. The radiant energy absorbed by the blackened strip increases the temperature of the strip. This changes its electrical resistance and destroys the balance of the bridge so that a sensitive galvanometer will be deflected from its original position.

A bolometer circuit is shown in Fig. 8·17. *A* and *B* are the bolometer filaments, R_1 and R_2 are resistances, and *G* is a galvanometer. R_3 is a shunt resistor used to zero the galvanometer.

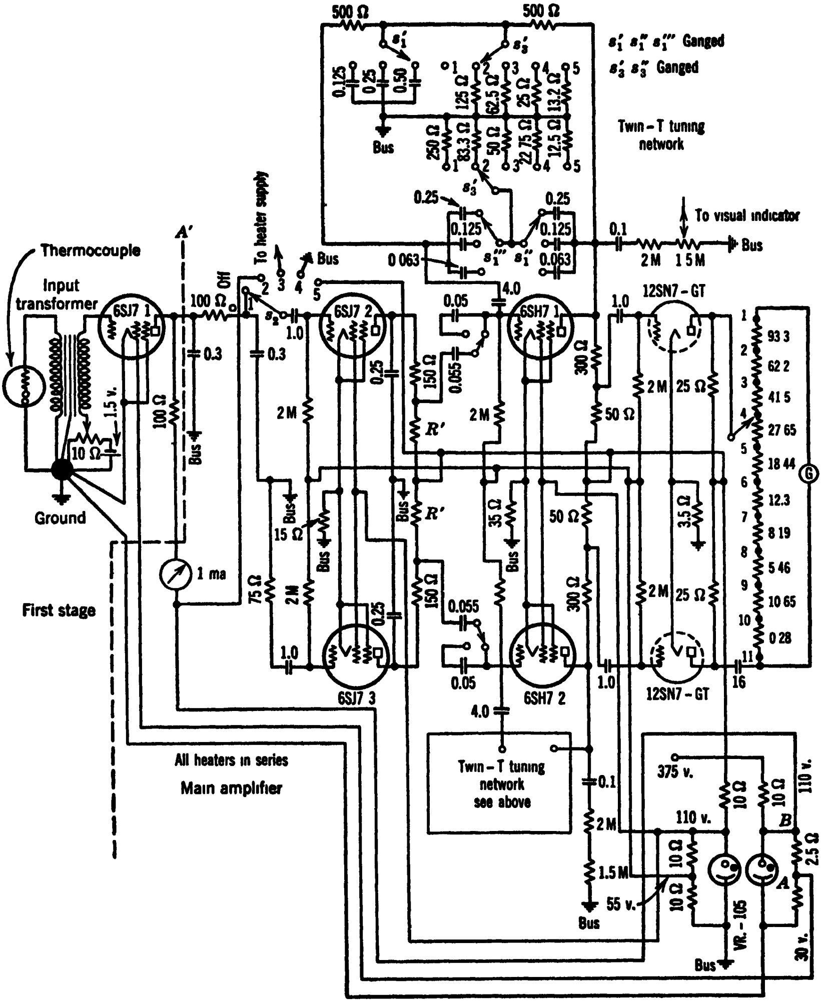


FIG. 8·16. Thermocouple amplifier schematic wiring diagram. (Courtesy of L. C. Roess and the publishers of *Review of Scientific Instruments.*)

In early work the bolometer was difficult to manipulate because of very large uncontrolled drift of the galvanometer. As a consequence most spectroscopists preferred to use the thermocouple in preference to the bolometer. However, many improvements, mainly introduced by Abbot, have now made the bolometer an easily handled instrument.

The simple expression given in equation (8·58) for the rate at which radiant energy is absorbed by the thermocouple receiver cannot be used to study the absorption of radiant energy incident on the bolometer filament. This is due to the fact that the filament is heated by the current passed through it. If Newton's law of cooling is assumed to be valid, the thermal equilibrium condition for a filament carrying current can be expressed as

$$RI^2 = K(T - T_a) \tag{8·69}$$

where R is the resistance of the filament, I is the current through the filament, T is its temperature, and T_a is the ambient temperature. The resistance R at any temperature T is related to the resistance R_s at some reference temperature T_s as follows:

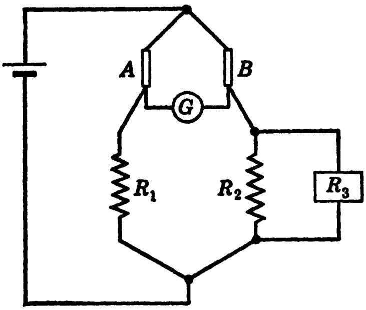


FIG. 8·17. A bolometer circuit.

$$R = R_s[1 + a(T - T_s)] \tag{8·70}$$

in which a is the temperature coefficient of resistivity. The resistance R_s is a reference resistance at zero current and at any one ambient temperature. If the ambient temperature is changed, R_s will vary according to equation (8·70). For convenience this equation may be rewritten with changed subscripts as

$$R_s = R_0[1 + a(T_s - T_0)] \tag{8·71}$$

where R_0 represents the resistance at zero current at a fixed reference temperature T_0. The temperature difference $T_s - T_0$ is now replaced by ΔT, and $R_s - R_0$ becomes ΔR. Then

$$\Delta R = aR_0 \, \Delta T \tag{8·72}$$

Moreover,

$$\Delta E = I \, \Delta R \tag{8·73}$$

Here ΔE is the potential change over the filament resulting from the change of resistance. Now the sensitivity of the bolometer is expressed in terms of the volts output per watt of radiant energy incident on the filament, that is, $\Delta E/RI^2$. Because

$$\frac{\Delta E}{RI^2} = \frac{IaR_0 \, \Delta T}{K(T - T_a)} \tag{8·74}$$

which if T_0 has been chosen properly becomes

$$\frac{\Delta E}{RI^2} = R_0AI \tag{8·75}$$

where $A = a/K$, it appears that the sensitivity may be increased proportionally by increasing the current through the bolometer. This is true provided the heating effect of the current does not raise the temperature of the filament more than about 15°C. above the surrounding temperature. Practically no increase in sensitivity is obtained from increases in bolometer current when the bolometer is heated more than about 25°C. above its surroundings.

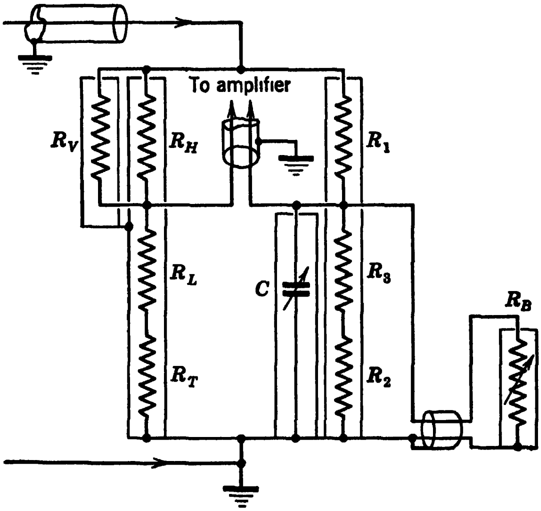
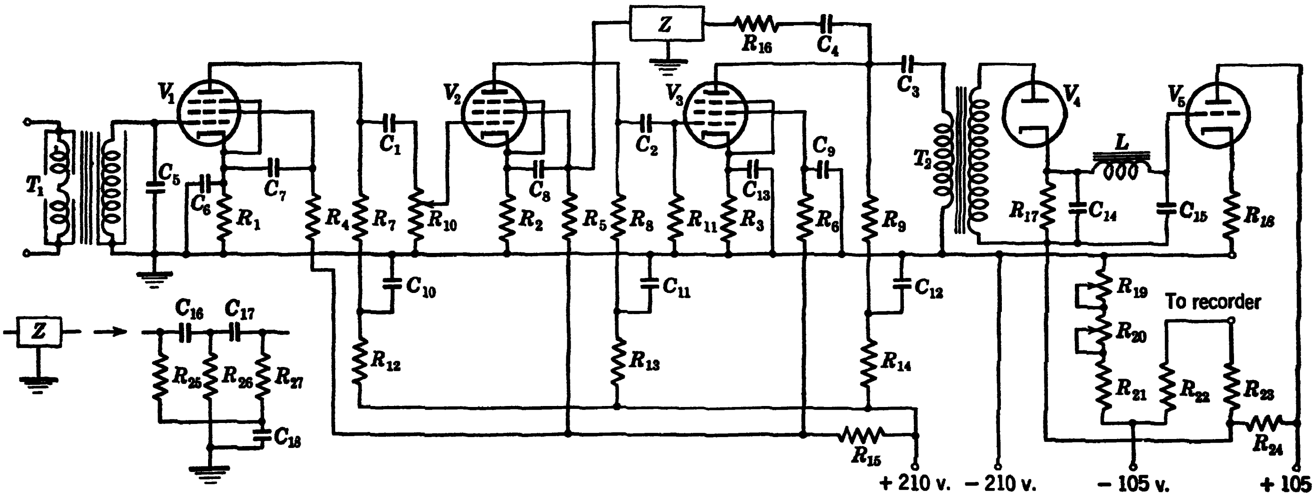


FIG. 8·18. Bolometer bridge circuit. (Courtesy of C. H. Schlesman, F. G. Brockman, and the American Institute of Physics.)

When a bolometer is evacuated, its sensitivity is increased about ten fold, all other conditions remaining the same. This difference in sensitivity is substantially the same as that observed for identical thermocouples operating under atmospheric and vacuum conditions, respectively. Unfortunately, the bolometer current under vacuum conditions must be reduced to one-fifth the amount used at atmospheric pressure to keep the filament within 15°C. of its surroundings. Therefore, the sensitivity of the vacuum bolometer is only $^{10}/_{5}$ or two times as sensitive as the bolometer operated at atmospheric pressure. In addition, the instrument used at atmospheric pressure is faster in response than the vacuum bolometer. These considerations, then, show that little is to be gained by operating a bolometer below atmospheric pressure, and as a result the high-vacuum systems necessary for the operating of thermocouples can be eliminated if bolometers are used as the detecting device for infrared radiant energy.



R_1, R_2, R_3	2,200 ohms	R_{22}	200 ohms	C_{16}, C_{17}	0.00300 mfd.
R_4, R_5, R_6	1.5 megohms	R_{23}	5,000 "	C_{18}	0.00600 "
R_7, R_8, R_9, R_{15}	0.5 megohm	R_{24}	25,000 "	T_1	Shielded, tuned secondary, turns ratio 1:30
R_{10}, R_{11}	1.0 "	R_{25}, R_{27}	53,000 "		
R_{12}, R_{13}, R_{14}, R_{16}	0.1 "	R_{26}	26,500 "	T_2	Turns ratio 1:30
R_{17}	2.0 megohms	C_1, C_2, C_3	0.003 mfd.	L	700 henries
R_{18}	5,000 ohms	C_4	0.1 "	V_1, V_2, V_3	Type 1221
R_{19}	10 "	C_5	0.024 "	V_4	6H6, diodes in parallel
R_{20}	100 "	C_6, C_{10}, C_{11}, C_{12}, C_{13}, C_{15}	2.0 "	V_5	6J5
R_{21}	100 "	C_7, C_8, C_9	0.05 "	All heaters supplied with 6.3 volts, 60 cycles.	
		C_{14}	0.05 "		

FIG. 8·19. Amplifier for alternating-current bolometer. (Courtesy of C. H. Schlesman, F. G. Brockman, and the American Institute of Physics.)

Schlesman and Brockman (*55*) have developed an alternating-current bolometer and amplifier for use in infrared spectroscopy. It is essential that their bolometer bridge, shown in Fig. 8·18, be shielded, since it is supplied with alternating current having a frequency of 1000 c.p.s.

The circuit diagram of the instrument used to amplify the output of the bridge is illustrated in Fig. 8·19. It is seen that a resistance-capacity T-network tuned to 1000 c.p.s. is used to limit as far as possible the response of the amplifier to the frequency supplied to the bridge.

Full-scale sensitivity at maximum gain is 0·13 microvolt when operated into a high-speed recorder with a 15-ohm input resistance and a full scale of 7.5 millivolts. When properly adjusted, the response is linear. Unfortunately, the useful sensitivity is limited by fluctuations originating in the power supply to 1.1 microvolts input for full-scale response of the output recorder. In addition such an installation allows considerable wandering of the zero unless adequate precautions are taken. All metal type bolometers are sensitive to mechanical vibration.

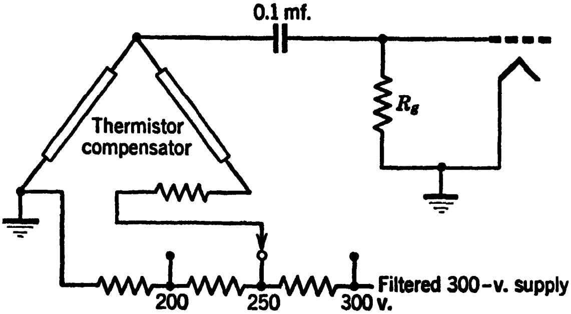


FIG. 8·20. Schematic diagram of input circuit.

In 1945 J. A. Becker of the Bell Telephone Laboratories disclosed to the members of the Optical Society of America at their Cleveland meeting the use of thermistor type bolometers for infrared spectroscopy. An amplifier developed for use with the bolometer was also described. This amplifier furnishes a d-c. electrical bias to the thermistor bolometer. The bolometer impresses a pulsating d-c. signal on the grid of the first amplifier tube because of the fact that the energy beam incident on the thermistor element is interrupted at a frequency of 15 c.p.s. The output of the first stage of amplification is further amplified in a narrow-pass-band tuned resistance-capacity coupled amplifier. The instrument is sharply tuned to 15 cycles by means of two parallel T-networks. The amplified signal is rectified to give a deflection on a d-c. meter or recorder. In Fig. 8·20 is shown the schematic diagram of the input circuit.

The complete wiring diagram, as modified by the author, is given in Fig. 8·21.

Five stages of amplification are provided. A three-stage unit employing RCA 1603, RCA 77, and RCA 6SJ7 tubes provides the initial amplification. This unit is provided with a negative feedback path containing a parallel-T-network adjusted for maximum loss at 15 cycles. Such an arrangement provides a minimum of feedback at this frequency, with

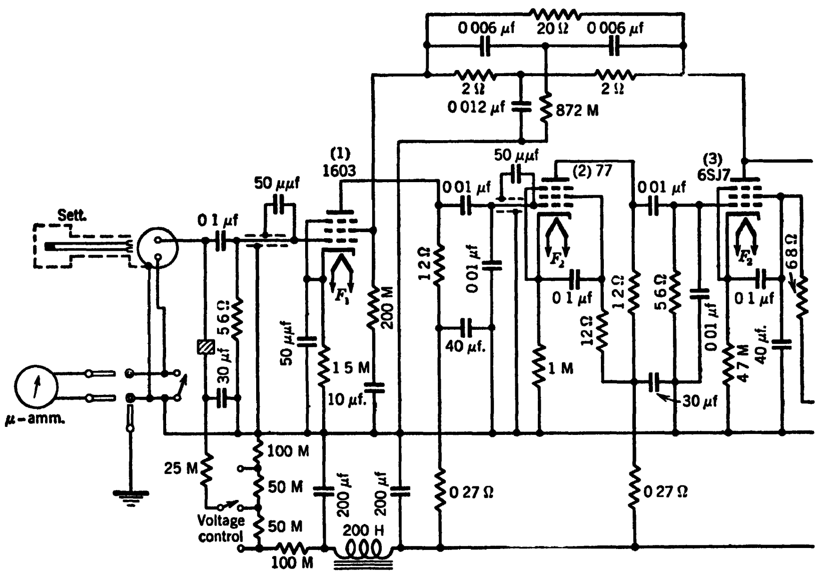
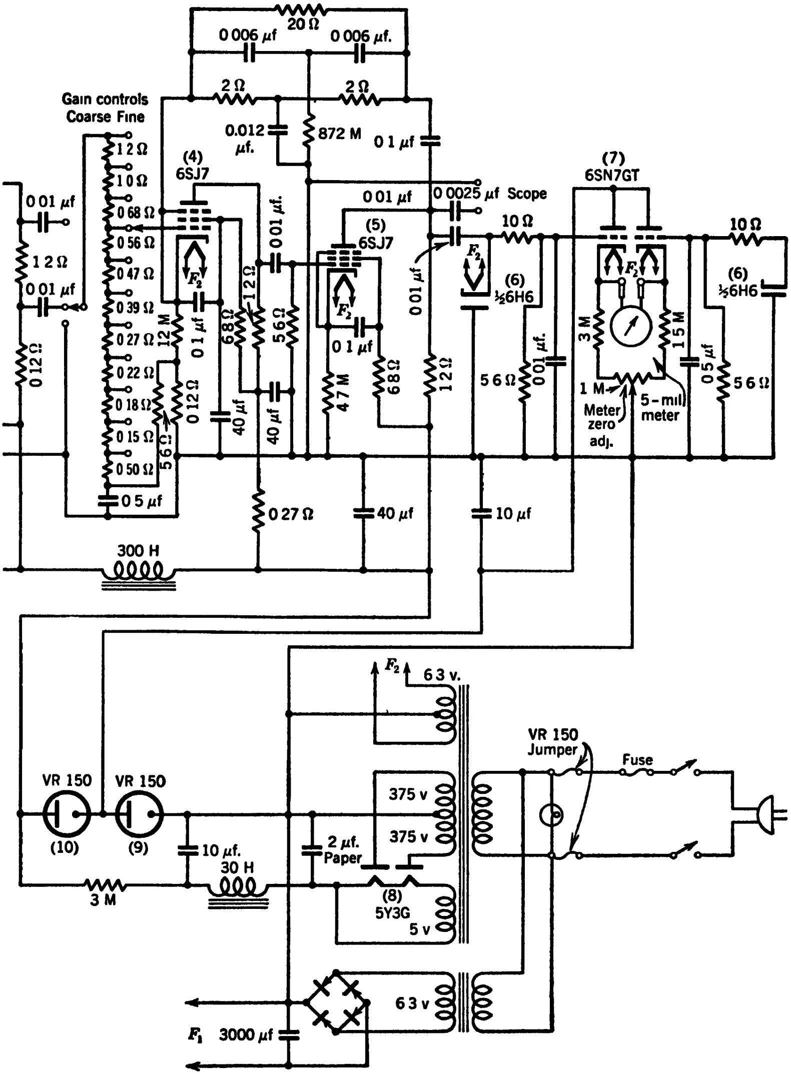


FIG. 8·21. Wiring diagram of 15-cycle ampli rapidly increasing feedback at frequencies above and below 15 cycles and hence decreased gain. On this is dependent the selectivity of the amplifier against noise of other than signal frequency. The feedback, while a minimum at 15 cycles, tends to reduce the over-all gain, but at the same time it insures a high degree of stability. Following this part of the amplifier are the gain controls and a two-stage amplifier employing 6SJ7 tubes. Here also a parallel-T feedback network is employed.



fier. (Courtesy of Air Reduction Company, Inc.)

After the final stage of amplification, the signal is rectified with one-half of an RCA 6H6 twin diode tube. Since this is operated at high input levels and with a large load resistance, linear response is achieved from 10 to 100 per cent full-scale deflection, using a 5-milliampere output meter to measure the deflections. The d-c. voltage derived from the rectifier is applied to one of the grids of a balanced cathode follower type of d-c. amplifier using an RCA 6SN7GT tube. The other grid of the d-c. amplifier is connected to a dummy rectifier circuit employing the other half of the 6H6 and having the same circuit constants as the actual rectifier. By means of this arrangement, drift due to either the rectifier or the d-c. amplifier is practically eliminated.

The sensitivity is such that 5 milliamperes output is obtained for an input of 2.3 microvolts. The gain is peaked at 15 cycles with an equivalent band width of 1.9 cycles. Amplifier noise is equivalent to an r.m.s. input of about 0.03 microvolt; hence the noise of the amplifier is below the Johnson noise level of the thermistor bolometer. After an initial warm-up period the drift is very small under normal conditions. If, however, the amplifier is in a room where the humidity is high, the warm-up period is lengthened; and if the humidity is very high, the amplifier will not function. This is due to current leakage over the high-resistance components. Although the amplifier is tuned to 15 cycles, adequate shielding is essential to preclude disturbances from all ordinary field conditions.

The air-backed thermistor bolometer and the vacuum thermocouple have about the same sensitivity to radiant energy in the near infrared portion of the spectrum up to about 6 microns. From about 6 to 8 microns the thermistor bolometer, not having a blackened surface, does not absorb energy efficiently; as a consequence, in this wavelength interval, the thermocouple is more sensitive. Beyond 8 microns the relative sensitivities of the two detectors are about equal. The thermistor bolometer, because it operates at atmospheric pressure, is not affected by mechanical vibrations; because of its high speed of response, about 30 milliseconds, it has much to recommend its use in infrared spectroscopy.

8·17 Prism spectrometers. Research infrared spectrometers used for developing analytical and control procedures are designed to provide the maximum resolution obtainable consistent with ease of operation, ruggedness, and reproducibility. Such work demands an instrument capable of covering a wide wavelength range (1 to 15 microns or more). Accurate measurement of absorbancies is at least as important as accurate wavelength determinations and high resolution. To cover the required wavelength interval with a grating spectrometer would require several gratings and provisions for changing them. Such an instrument would be overelaborate in design.

At present research type infrared spectrometers are more or less custom built to fit the needs of individuals. Because of the diverse requirements of spectroscopists, the resulting instruments differ considerably. For example, an instrument used at E. I. duPont de Nemours and Company is arranged so that either a prism or a grating may be used (*19*). In a spectrometer described by Brattain (*10*), "the prism is turned by a cam arrangement at such a rate that regardless of the variation of spectral slit width with prism position and regardless of the variation of actual slit width necessary to maintain constant energy, the prism turns through each spectral slit width in constant time." A modification of the instrument originally described by McAlister, Matheson, and Sweeney (*37*) makes it possible to record absorbancies (optical densities) directly with an accuracy of better than 2 per cent (*58*). Various other methods are employed for producing spectrograms on which either or both zero and 100 per cent transmittance values are recorded (*6*, *10*, *60*). These permit the determination of the percentage transmittance as a function of wavelength. Oetjen (*42*) has described a versatile instrument which permits the use of any one of two prisms and two gratings.

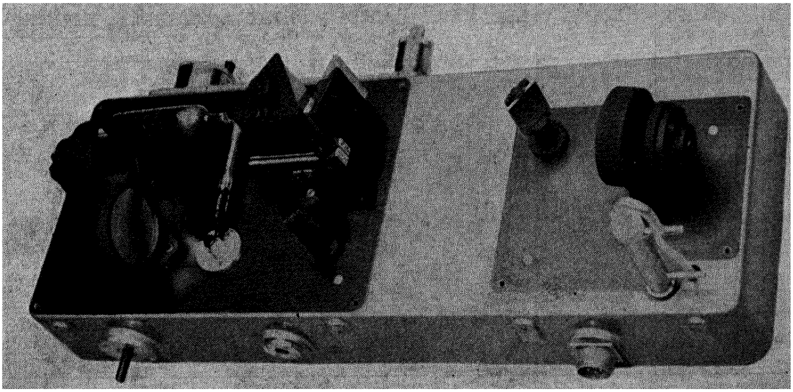


FIG. 8·22. Top view of Perkin-Elmer spectrometer with covers removed. (Courtesy of the Perkin-Elmer Corporation.)

Fundamentally all infrared spectrometers are similar, differing only in detail. In Fig. 8·22 is shown the top view of the Perkin-Elmer Model 12A infrared spectrometer with covers removed.

Here the source is a short Globar with silvered ends to insure good electrical contact, mounted in a water-jacketed housing. The slits are bilateral, simultaneously operated, and made so that the slit widths can be accurately reproduced. The entrance slit is curved in order to produce a straight image at the exit slit. An 18° off-axis paraboloid mirror, accurately figured, is used as a collimator for the 60° prism. This mirror has a focal length of 27 cm. and a diameter of 8 cm. This allows prisms as large as 60-mm. height × 75-mm. base to be used, and provides an effective aperture ratio of *f*:4.5. Prisms and automatic temperature-compensation devices are mounted on tables which can be interchanged rapidly and locked into position accurately, making it possible to change prisms at will. The receiver is a compensated vacuum type thermocouple, evacuated and the vacuum made semipermanent by means of a charcoal "getter." The amount of radiant energy falling on the thermocouple can be measured by means of a high-sensitivity galvanometer, but for plant control work it is often desirable to amplify the thermocouple output and to measure the amplified e.m.f.

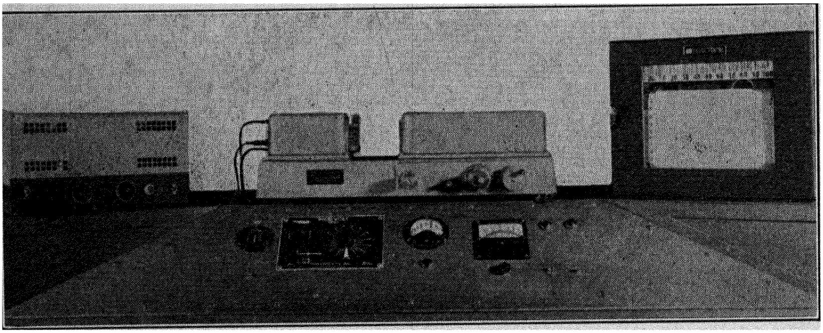


FIG. 8·23. Spectrometer assembly. (Courtesy of Air Reduction Company, Inc.)

In Fig. 8·23 is shown a Perkin-Elmer spectrometer mounted in a special cabinet which also houses an amplifier for the output of the thermistor bolometer which is employed in place of the more commonly used thermocouple. Provision is made for scanning the spectrum continuously, and the radiant energy falling on the detecting device is recorded with an electronic recorder. The installation is completely a-c. operated.

In Fig. 8·24 is shown the schematic optical path of a Beckman Model IR2 infrared spectrophotometer. The source *A* is a water-jacketed, self-starting Nernst Glower monitored by the phototube *R* which actuates an electronic regulator, thereby maintaining the energy output constant to within 0.1 per cent. The beam is interrupted ten times per second with the revolving sector *B*, passes through the window

C, and is focused by the concave mirror *D*. The concave rock-salt lens *E* renders the beam from *D* parallel for passage through the liquid absorption cell *G*. *F* is a filter slide. Two 10-cm. gas cells, *I*, are arranged so that either cell can be placed in the light path. Lenses *H* and *K* focus the beam on the upper entrance slit *L* of the monochromator. A spherical mirror *M* with a radius of 100 cm. is used as a collimator for a 60°, 60-mm. base prism. This mirror also focuses the dispersed beam on the exit slit *L* which is located directly under the entrance slit. By proper setting of the Littrow mirror *O*, a beam of selected wavelength passes through the exit slit, from where it is directed by the two mirrors *J* and *P* onto the receiver element in its housing *Q*.

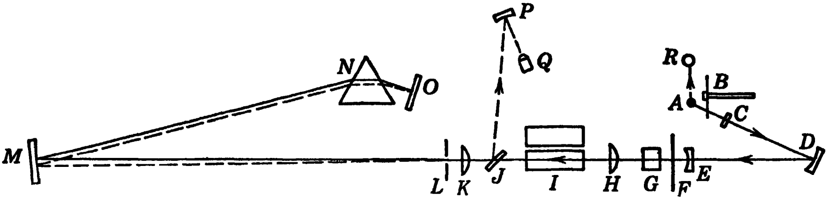


Fig. 8·24. Schematic optical path of Beckman Model IR2 infrared spectrophotometer. (Courtesy of National Technical Laboratories.)

Electrical energy from the receiver is amplified with a special 10-cycle amplifier and the amplified current recorded with an electronic recorder. Provisions are also made for obtaining transmission values directly in terms of either percentage transmittance or absorbance. The change of prisms is effected by changing monochromators. Figure 8·25 shows the instrument, amplifier, recorder, and gas-handling system all mounted on a special table.

In Fig. 8·26 is shown a schematic diagram of the Baird Associates infrared recording spectrophotometer. This instrument utilizes a rugged but sensitive bolometer as a servo-control. It detects lack of balance in the intensities of two infrared beams which illuminate it alternately. The source of infrared energy is a Globar mounted in a water-cooled housing. Two beams from this source are focused by concave mirrors onto the entrance slit of the spectrophotometer in such a manner that the optical paths are symmetrical. In one of these beams the sample is placed, and in the other beam a compensating cell is placed. A 10-cycle interrupter chops the two beams and causes them to illuminate the spectrophotometer slits alternately.

The beam passes through the slit and is collimated with a spherical mirror having a radius of curvature of 150 cm. It is used 4° off axis. The radiant energy is dispersed with a 60° rock-salt prism having a

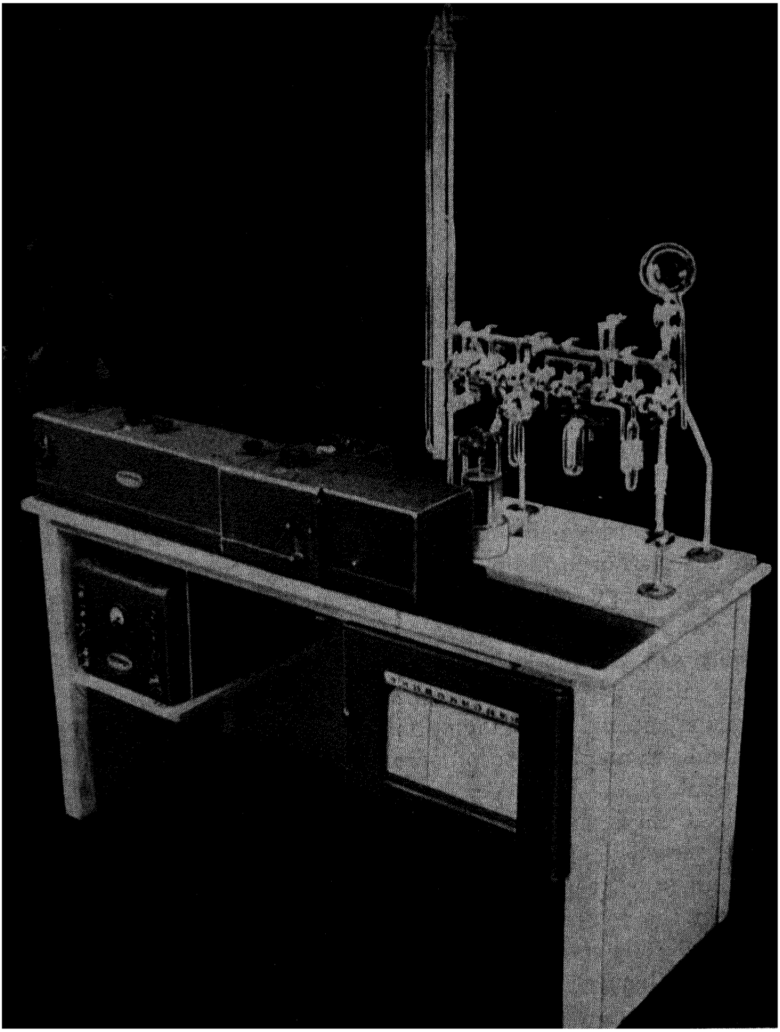


Fig. 8·25. Beckman infrared spectrophotometer assembly. (Courtesy of National Technical Laboratories.)

10-cm. base and 8-cm. height. The Littrow mirror is cam driven in such a way that a linear wavelength scale is produced. A cam drive, mechanically coupled to the slits and to the wavelength drive, control the width of the slits in such a way that essentially constant radiant energy passes through the slits throughout the spectrum when the sample cell is empty.

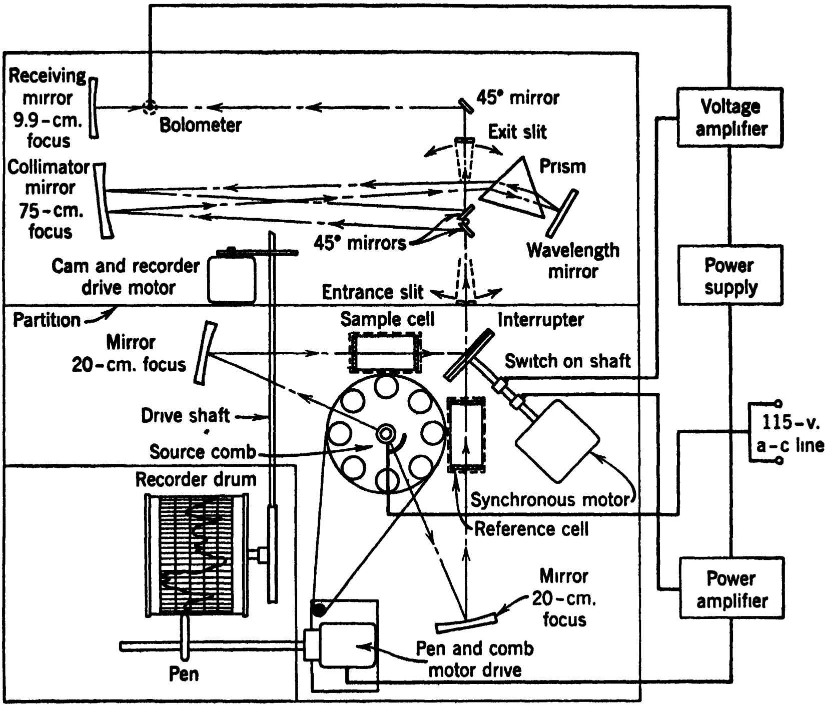


FIG. 8·26. Schematic diagram of the Baird Associates recording spectrophotometer. (Courtesy of Baird Associates, Inc.)

The rotation of the recorder is synchronized mechanically with the rotation of the Littrow mirror. A comb-shaped diaphragm near the Globar is driven by the amplified bolometer signal in such a way that it trims the beam passing through the reference cell and equalizes it with the beam passing through the sample cell. The comb is coupled to the pen drive so that a continuous and linear record of the transmittancy of the sample is obtained.

Two amplifiers are used in conjunction with this instrument, a bolometer amplifier and a recorder amplifier.

The bolometer amplifier has a gain of approximately 5×10^7 and an inherent noise level of about 5×10^{-9} volt which is the same order of magnitude as the bolometer noise. At 100 per cent transmittancy, signals of the order of 1 microvolt are obtained. A four-stage, resistance-coupled circuit tuned to 10 c.p.s. is used. For one-half of maximum gain the band pass is about 5 c.p.s. The power supply consists of a 300-milliampere filament supply, with 60 to 120 cycle ripple less than 2 per cent; a plate supply of 250 volts, with ripple as supplied to first tube less than 0.1 per cent; and a voltage regulator holding the 110-volt input to ±0.5 per cent for line fluctuations of ±10 per cent.

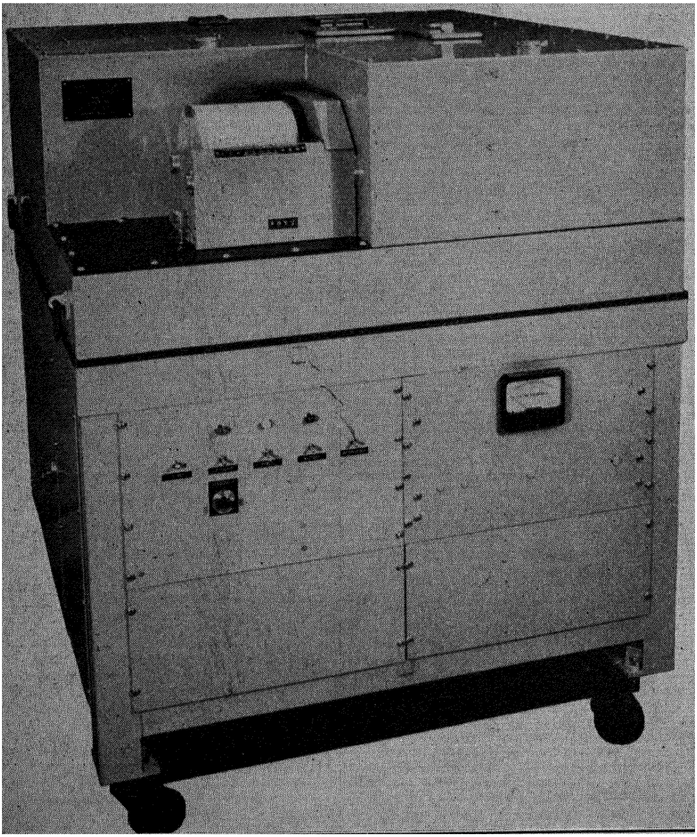


FIG. 8·27. Baird Associates recording infrared spectrophotometer. (Courtesy of Baird Associates, Inc.)

The recorder is driven by a 60-c.p.s. motor powered from a three-stage d-c. power amplifier. This has an effective gain of approximately 50, and a noise level of less than 0.3 volt. The input is the 10-c.p.s.

output current of the voltage amplifier after rectification by the commutator of the light beam interrupter. The power supply is self-contained.

In Fig. 8·27 is shown the complete spectrophotometer. This instrument has the following characteristics. It has a high degree of stability and is free from drift. Since it records absorption curves directly with a precision of ±0.5 per cent throughout its range from 2 to 16 microns in 12 minutes, it has distinct advantages. The disadvantages associated with it are lack of resolution and means for determining when the radiant energy in both the sample and reference beams is too low to allow the instrument to function properly.

8·18 Grating spectrometers. Where higher dispersion and resolution are required in the infrared, as for the examination of the fine structure of bands or for the accurate measurement of wavelengths, grating spectrometers are used. One difficulty of the grating is that higher orders of short wavelengths overlap the long wavelengths of lower order. This characteristic is especially troublesome in the infrared region of the spectrum because the sources which are used are always much richer in near infrared and visible radiant energy than in infrared. For isolating certain regions filters or shutters with selective transmittance may be used, but not many substances are available which cut off sharply at the desired wavelengths (*48*). An expedient which is commonly used in the near infrared is that introduced by Randall, who placed a prism spectrometer of low dispersion before the grating instrument. The prism disperses the radiant energy, and a narrow band of the resulting spectrum is allowed to fall on the entrance slit of the grating spectrometer.

The first instrument utilizing this arrangement was developed by Sleator (*56*) under Randall's direction and is shown schematically in Fig. 8·28.

Energy from the Nernst glower L is focused on the slit S_1, then collimated by the spherical mirror M_2, and directed toward an 18° rocksalt prism P. Provisions are made for rotating the prism P and plane mirror M_4 about K simultaneously so that any region of the spectrum may be isolated for the grating, thus avoiding overlapping of spectra. Radiant energy of a selected frequency range, including not more than one octave, passes through slit S_2 to the spherical mirror M_3 which serves as a collimator for the grating G. A narrow frequency band of the highly dispersed beam returns nearly on its own path and is focused by the spherical mirror M_3 on the thermocouple slit C. Bands of different frequencies are swept across the slit by rotating the grating. Since the entrance and exit slits cannot be coincident, the spherical mirrors are used slightly off their axes, and a certain amount of astigmatism results. By increasing the size of the mirrors and their focal lengths, the effective aperture remaining constant, this trouble can be reduced somewhat, since the minimum angles between the beams and the mirror axes may then be made smaller. However, a better method of avoiding astigmatism, one which allows the slits to be placed on the mirror axes, was suggested by Pfund (*46*). The optical arrangement of an instrument utilizing his system was set up by Hardy (*25*) at the University of Michigan and is shown schematically in Fig. 8·29.

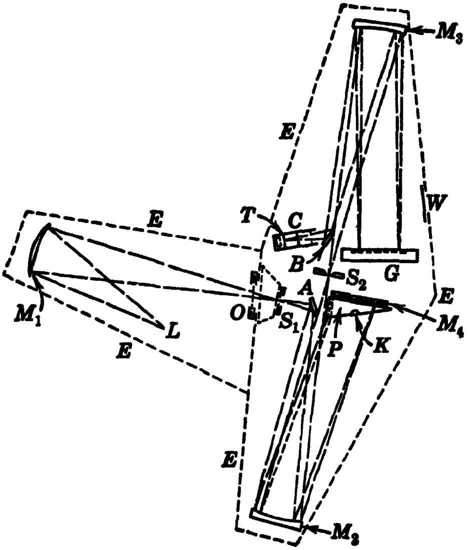


Fig. 8·28. Prism-grating combination spectrometer.

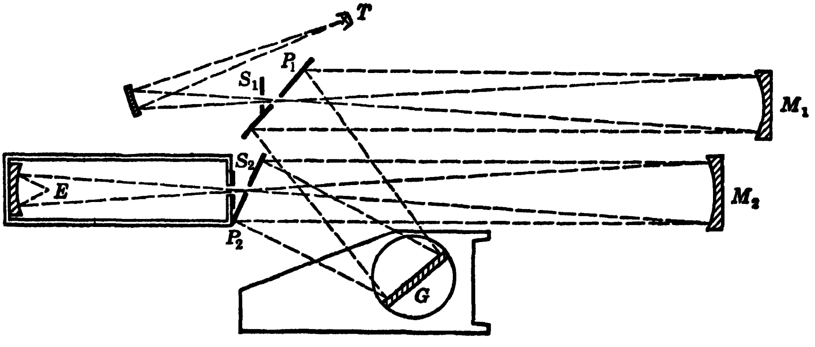


Fig. 8·29. Large infrared grating spectrograph with Pfund mirror arrangement.

Radiant energy from a Nernst glower T is diffracted by a rock-salt fore-prism which is not shown. Energy of a selected frequency range from the fore-prism is allowed to fall on the entrance slit S_1, thence passes through a slot in the center of the plane mirror P_1. The slits S_1 and S_2 are both placed on the optical axes of the parabolic collimating mirrors M_1 and M_2. As a consequence, a parallel beam from M_1 is returned to the plane mirror P_1 and reflected toward the grating without astigmatism. After diffraction by the grating, the light follows an inverse path to the parabolic mirror M_2, which focuses a narrow frequency band on the exit slit S_2. An elliptical mirror, with the conjugate focii located at the exit slit and the thermocouple E, respectively, focuses a reduced image of the exit slit on the receiver of the vacuum thermocouple.

The number of reflections involved in this system is not a serious handicap because of the high reflecting power of metallized surfaces for infrared radiant energy. Even badly tarnished aluminized mirrors function satisfactorily at wavelengths longer than 5 microns.

The grating used in the near infrared is a 5-inch plane grating with 15,000 lines to the inch. With this a resolving power of nearly 1000 is attained. This is, of course, only a small fraction of the theoretical resolving power of 75,000, the difference being ascribed to the wide slits which must be used for adequate illumination.

8·19 Other infrared gas analyzers. In addition to the conventional spectrometers, differential analyzers have been developed by Pfund (*50*) and Wright (*59*). These instruments may be described as spectrometers of high resolving power and zero dispersion. They are of particular value in the petroleum, chemical, and allied industries wherever a continuous analysis of a component in a gas or liquid stream (nonaqueous) is desired.

In Fig. 8·30 is shown the optical diagram of the infrared analyzer manufactured by Baird Associates, Cambridge, Massachusetts.

The ultimate sensitivity of the control type analyzer is better than one part of carbon monoxide in 5000 parts of air. The precision obtainable depends to a large extent on the nature of the gas system, the maximum being limited by the output meter or recorder.

The principle of this analyzer involves two balanced infrared beams from a single source. They are focused by front surface mirrors on bolometer arms which are elements of a Wheatstone bridge. Any difference in the energy incident on the bolometers unbalances the bridge, and the amount of off-balance voltage (or resistance change necessary to restore balance) is a measure of the quantity of the absorbing gas in the sample.

To operate the analyzer, a stream of gas is passed at constant pressure through a sample cell that covers both infrared beams. In addition, a relatively high concentration of the gas to be measured is maintained in the filter cell. This cell covers only one infrared beam and one bolometer. Any absorption of the energy by the various components of the sample has a differential effect on the bolometer elements only when the absorption occurs in the portion of the spectrum in which the filter gas absorbs.

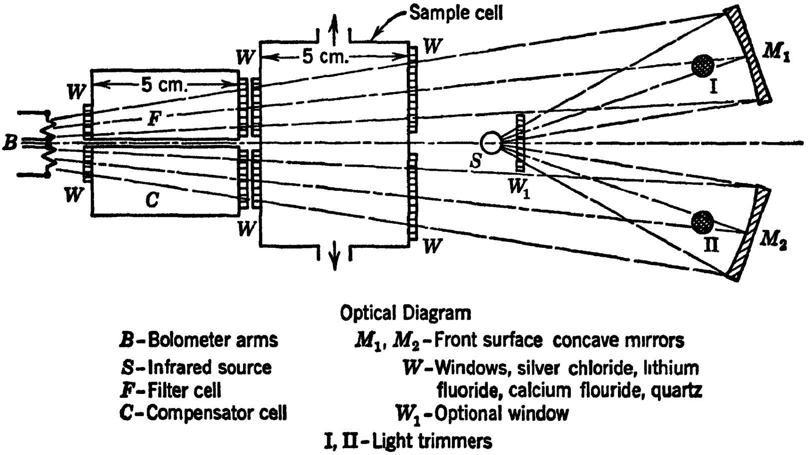


FIG. 8·30. Infrared analyzer—double cell unit. (Courtesy of Baird Associates, Inc.)

For complicated gas systems containing constituents with overlapping bands, filters of these interfering gases are inserted into both beams. This gives the instrument very high discriminating power.

A knowledge of the concentrations of all the components of the gas system is essential for optimum use of these analyzers. Before an analyzer is selected for a definite application a thorough analysis by other methods is desirable. It is then possible to select the proper model. All gases must be at room temperature and present at pressures well below their vapor pressure at this temperature.

8·20 Wavelength calibration. Prism spectrometers must be calibrated by determining the positions of the wavelength dial or micrometer screw at which the absorption bands of several common substances may be found. It is essential that only materials whose absorption bands have been fixed precisely with grating spectrometers be used as calibration standards. It is desirable to have absorption bands that are sharp, easily recognized, and found at frequent intervals throughout the range of the spectrometer. Table 8·5 gives a list of some of the substances which best fulfill these requirements as well as the particular wavelength interval in which each is used.

TABLE 8·5 SUBSTANCES USED FOR WAVELENGTH STANDARDS FROM 0.5 TO 25 MICRONS

Substance	*Wavelength Interval*
Sodium arc	0.5890
Mercury arc	0.5460–1.53
Atmospheric CO_2 and water vapor	2.58 –2.77
Ammonia	2.85 –3.17
Methane	3.17 –3.47
Atmospheric CO_2	4.22, 4.28
Atmospheric water vapor	5.00 –7.85
Ammonia	7.9 –14.05
CO_2	13.85 –15.1
Methyl alcohol	12.2 –24.0

Absorption and emission curves of these materials are available in the literature as follows:

Na and Hg arc: *Handbook of Chemistry and Physics*, Chemical Rubber Publishing Co., Cleveland.
CO_2: E. F. Barker, *Astrophys. J.*, **55**, 391 (1922).
H_2O: R. A. Oetjen, Chao-Lan Kao, and H. M. Randall, *Rev. Sci. Instruments*, **13**, 515 (1942).
NH_3: G. A. Stinchcomb and E. F. Barker, *Phys. Rev.*, **33**, 305 (1929).
CH_4: A. H. Nielsen and H. H. Nielsen, *Phys. Rev.*, **48**, 864 (1935).
CH_3OH: A. Borden and E. F. Barker, *J. Chem. Phys.*, **6**, 553 (1938).

The micrometer setting of the instrument should be plotted against the wavelength of as many absorption bands as are considered necessary. This plot should be on a large piece of graph paper, and the ordinates should be adjusted so that the resultant curve will have a slope of about 45°. The curve is smoothed, using standard procedures.

Frequent checks of one or more characteristic bands will indicate the extent and nature of the recalibration required to maintain an accurate calibration curve. Since the last traces of carbon dioxide and water vapor are removed from the spectrometer only with difficulty, these serve as convenient standards for rechecking the instrument.

8·21 Qualitative analysis. It was pointed out in Section 8·2 that not only the molecule but also the functional group comprising the molecule often have characteristic absorption bands. As a consequence, it is frequently possible to identify the principal functional groups within the molecule. Such information can be used positively to identify compounds in cases where chemical evidence limits the material to relatively few possible structures. It is particularly valuable

TABLE 8·6 ABSORPTION BANDS FOR VARIOUS FUNCTIONAL GROUPS

Functional Group	*Position of Absorption Band, Microns*
H_2O (atm.)	2.58, 2.68
CO_2	2.69
CO_2 (atm.)	2.76
Asym. $-NH_2$	2.82–2.94
Bonded $-OH$ and $\overset{\vert}{\underset{\vert}{N}}-H$	2.84–2.99
Sym. $-NH_2$	2.87–2.99
$C{=}N-H$	2.96–2.99
$\equiv C-H$ (acetylenes)	3.02–3.10
$C-H$ (phenyl)	3.22–3.24, 3.22–3.28, 3.28–3.31
$C-H$ (olefins)	3.30–3.36
$-CH_3$	3.37–3.49
$\rangle CH_2$ sat.	3.42–3.52
$-\overset{\vert}{\underset{\vert}{C}}-H$ sat.	3.48–3.54
$S-H$	3.85–4.08
$-C\equiv N$ (nitrile)	4.16–4.55
CO_2 (atm.)	4.22 and 4.28
$-C\equiv C-$	4.46–4.61
$-C\equiv N\langle$	4.59–4.72
$\rangle C{=}O$ (carbonyl)	5.37–6.1
$C{=}O$ (anhydrides)	5.41–5.56, 5.56–5.71
term. double bond	5.44–5.54
$C{=}O$ (esters)	5.71–5.81
$C{=}O$ (acids)	5.75–5.98
$C{=}O$ (aldehydes and ketones)	5.76–5.98
$C{=}N$	6.04–6.21
$-NH_2$ (amine)	6.08–6.35
$C{=}C$ (aromatic ring)	6.17–6.30, 6.60–6.75
$C{=}C-C{=}C$ (full aliphatic conj.)	6.21–6.34
$-NO_2$ (nitro)	6.32–6.44
$=C=S$	6.45–6.63
$-\overset{\vert}{\underset{\vert}{C}}-Cl$	6.63–6.74
CH_2 and CH_3	6.78–7.03
$-CH{=}CH_2$	7.04–7.16
$-\overset{\vert}{\underset{\vert}{C}}-CH_3$ (methyl)	7.20–7.33
$C-F$	7.63–7.78
$=\underset{\vert}{C}O-$ (phthalates)	7.78–7.91, 8.85–9.02
$-\overset{\vert}{\underset{\vert}{C}}-O-$	9.05–9.17, 9.32–9.42

in indicating the type of compounds that might be present in an unknown. For example, if an unknown absorbs strongly in the 5.75- to 6.00-micron region, aldehydes or ketones may be present. Other tests both physical and chemical may be used to confirm the results.

In Table 8·6 are shown various functional groups and the regions in which they absorb. Care should always be used in assigning an absorption band to a given functional group, since it must be remembered that the molecule also has characteristic absorption bands.

8·22 Quantitative analysis. The actual physical quantity that is measured by a spectrometer is the intensity of energy within a narrow wavelength interval. To the first approximation all substances which absorb in the infrared region of the spectrum follow the familiar Beer-Bouguer law:

$$I = I_0 e^{-(a_s)bc} \tag{8·76}$$

where I_0 is the energy incident on the sample, I is the energy transmitted by the sample, a_s is the absorbancy index (see Chapter 2), c is the concentration, and b is the length of the optical path through the sample usually equal to the length of the absorption cell. This expression can be put in the alternate form:

$$\log_{10} \frac{I_0}{I} = \log_{10} \frac{1}{T} = a_s bc = A_s \tag{8·77}$$

where T is the transmittancy fraction, a_s is the absorbancy index, and A_s is the absorbancy (optical density). For monochromatic beams the absorbancy (optical density) of a mixture equals the sum of the absorbancies of the components. That is, at any wavelength λ,

$$(A_s)_m = (A_s)_1 + (A_s)_2 + (A_s)_3 + \cdots + (A_s)_n \tag{8·78}$$

where $(A_s)_m$ is the absorbancy of the mixture, and $(A_s)_n$ is the absorbancy of the nth component. In general, n such equations, one for each of n selected spectral positions, are required to determine the concentration of each component present.

Considerable care must be exercised in choosing the spectral positions to be used for analysis. Usually the first step in the choice of spectral positions is to plot on a single sheet of graph paper the transmittancy curves of all components in the mixture under consideration. From the composite a choice of the best spectral positions can be made, using the following rules:

1. The spectral positions are chosen so that at each wavelength only one of the components is the major absorber.

2. One such spectral position is chosen for each of the components in the mixture. Although it is possible to perform the analysis using one less spectral position than there are components to be determined by using the equation of composition of the sample, such a procedure is not recommended.

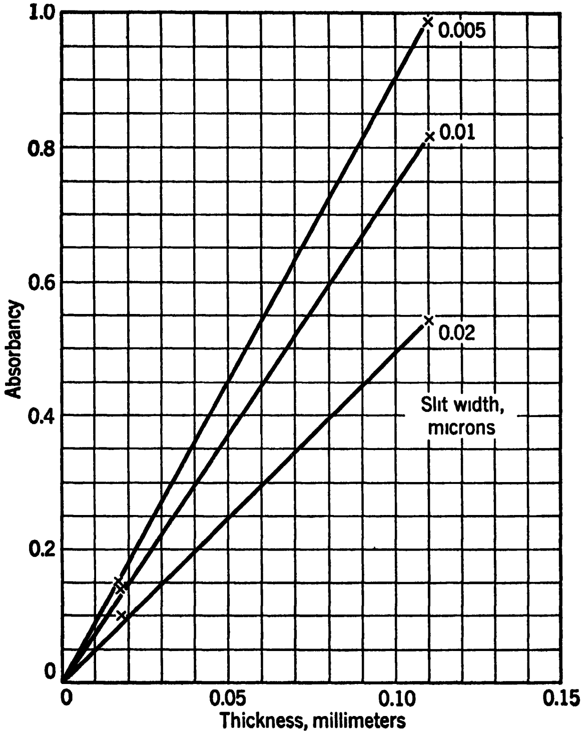


FIG. 8·31. Absorbancy of 7.99-μ band of isooctane as a function of cell thickness and slit width.

3. Whenever possible, the positions chosen should be at the peak of the absorption band of the major absorber. Unfortunately, this cannot always be adhered to. Where the side of a band must be used for analyses, the limiting factor in the accuracy of the analysis may be the reproducibility of the wavelength setting of the spectrometer.

4. It is advisable to choose spectral positions so that the absorbancies of the minor absorbers do not change by more than a factor of two over the spectral region subtended by the slit widths being used.

8·23 The choice of slit widths. In choosing the slit width for a given spectral position compromises must frequently be made. It is desirable to keep the slits as narrow as possible, especially when measurements are made on the side of an absorption band. In many cases narrow slits also reduce the deviations from the Beer-Bouguer law. On the other hand narrow slits limit the amount of energy that reaches the detecting system so that I and I_0 cannot be measured with the same accuracy as when the slits are wider. Where only broad absorption bands are being measured, the slit widths need be limited only by the mechanical characteristic of the spectrometer iteslf. But when narrow absorption bands are encountered or where absorption measurements are made on the side of a band, the slits should be as narrow as they can be made and still obtain reliable values for I_0 and I.

In Fig. 8·31 is shown the relation between the absorbancy of isooctane at 7.99 microns, the cell thickness, and the slit widths. The data were obtained with a Perkin-Elmer infrared spectrometer. It is obvious that, having made a choice of slit widths to be used at a given spectral position, this width should always be used for subsequent analysis.

8·24 The choice of cell lengths. The condition for greatest accuracy in absorption measurements is that the accuracy in the measurement of an absorbancy index shall have a maximum. This result can be achieved for gases by using an absorption cell of a fixed length and varying the gas pressure until the ideal concentration is reached. For liquids, however, the cell length must be chosen properly. Either a variable length cell (*23*) may be used or a cell having a fixed optical path within the desired range should be employed.

By stating that the accuracy in the measurement of an absorbancy index shall have a maximum it is implied that $\Delta A_s/A_s$ shall have a minimum value. Here A_s is the absorbancy, and ΔA_s is the smallest detectable change in the absorbancy. Since

$$2.3\,A_s = \log_e \frac{I_0}{I} \tag{8·79}$$

and

$$2.3\,\Delta A_s = \frac{-1}{I}\cdot \tag{8·80}$$

$$\frac{\Delta A_s}{A_s} = \frac{-1}{I_0(A_s)_e - (A_s)} \tag{8·81}$$

To find the optimum value of A_s equation (8·81) is differentiated and equated to zero. It is found that the absorbancy at which $\Delta A_s/A_s$ reaches a minimum is 0.4343, which corresponds to a transmittancy of 37 per cent.

If the absorbancy, A_s, is plotted against $A_s \cdot 10^{-A_s}$, it is seen that all absorbancies between 0.2 and 0.8 may be used in analytical work without any considerable loss of accuracy. This is shown in Fig. 8·32.

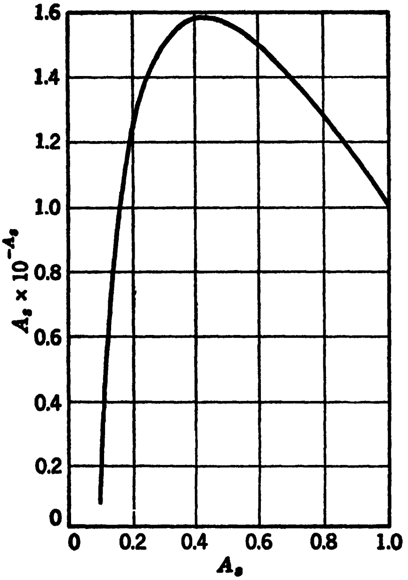


FIG. 8·32. Evaluation of the optimum absorbancy.

8·25 Determination of false energies. After one decides upon the spectral positions, slit widths, and gas pressures to be used for a given analysis, the next step is the determination of the false energy, if any, at each of the spectral positions. With a suitable combination of filters and shutters, it is possible practically to eliminate false energy in infrared instruments for wavelengths less than about 11 microns. Above this wavelength, however, it becomes appreciable in most instruments and must be taken into account.

Any light incident on the thermocouple which has a wavelength greater or less than the essentially monochromatic beam passing through the exit slit of the spectrometer may be termed false energy. Since the thermocouple responds equally well to light of all wavelengths, the intensities of both the incident and transmitted light as measured by the spectrometer will be too large by an amount equal to the false energy. For this reason the false energy at each spectral position chosen for analysis must be determined and subtracted from both the I_0 and I values in order to determine the transmittancy of the sample. Thus the corrected absorbancy, $(A_s)_c$, is given by

$$(A_s)_c = \log_{10} \frac{(I_0 - I_f)}{(I - I_f)} \tag{8·82}$$

where I_f is the false energy and is best expressed as a fraction, ϕ, of the incident radiant energy, I_0, as measured by the spectrometer, that is,

$$I_f = \phi I_0$$

The most satisfactory method of measuring the false energy at any wavelength is to place some substance which absorbs all radiant energy within the wavelength interval of interest, and then to note the amount of energy measured by the spectrometer. The amount so measured is I_f.

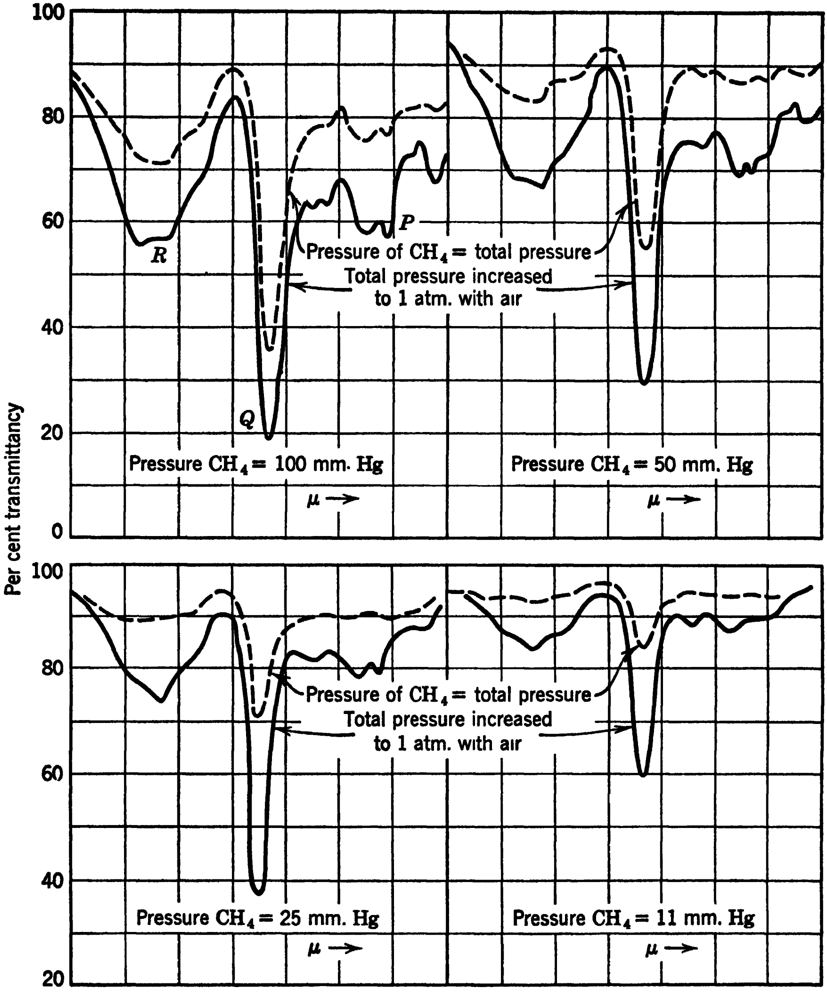


FIG. 8·33. The pressure dependency of the 7.58-μ band of methane.

8·26 One-component determinations. In many types of samples, the number of components is so limited and the absorption spectra are so characteristic that it is possible to determine the concentration of one of the components by measuring the transmittancy of the sample at a single wavelength. In most samples of this nature, the components are of such low molecular weight that they exhibit the phenomenon of changing their absorbancy indexes with pressure and diluents. As a result, most such determinations are empirical and are applicable only to the particular types of samples for which calibration data are ob-

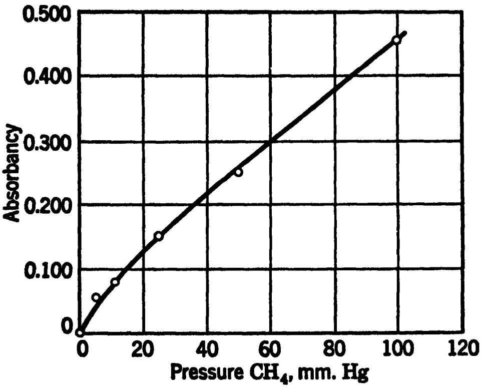


FIG. 8·34. The relation of absorbancy to the pressure of methane.

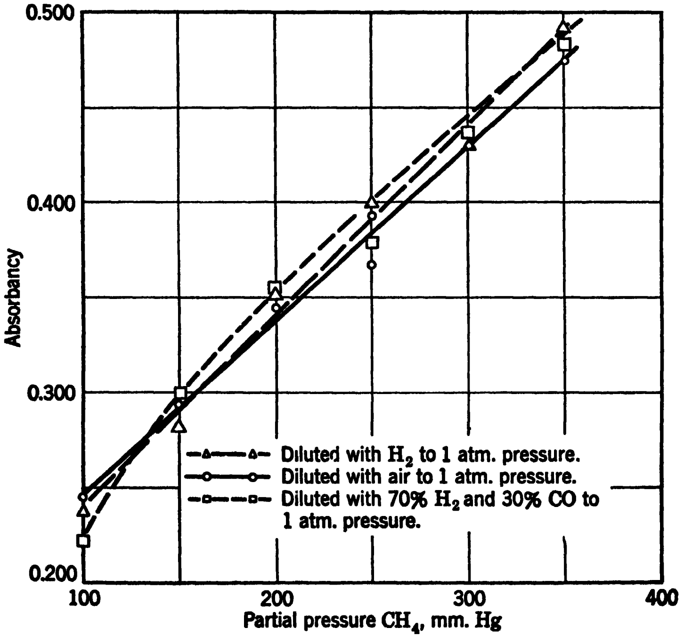


FIG. 8·35. The effect of various diluent gases on the absorbancy of the *P*-branch of the 7.58-μ band of methane.

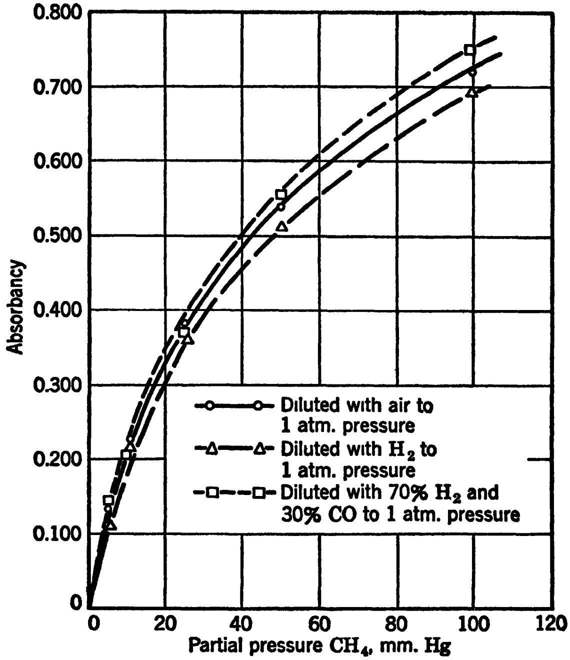


FIG. 8·36. The effect of various diluent gases on the absorbancy of the *Q*-branch of the 7.58-μ band of methane.

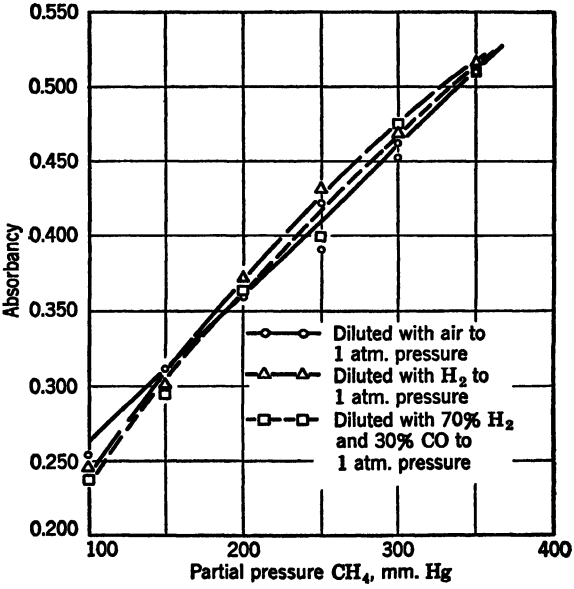


FIG. 8·37. The effect of various diluent gases on the absorbancy of the *R*-branch of the 7.58-μ band of methane.

tained. An example of this kind is afforded by the determination of methane in samples consisting predominantly of hydrogen and carbon monoxide.

In Fig. 8·33 is shown the pressure dependency of the 7.58-micron band of methane. Here the broken curve shows the transmittancy of

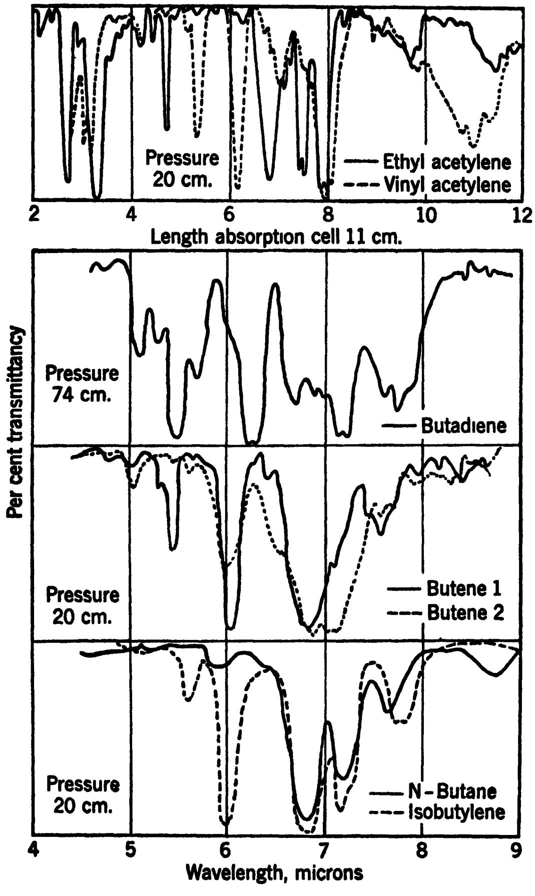


FIG. 8·38. Infrared transmittancy curves of butadiene and associated impurities.

methane when the pressure of methane equals the total pressure. The solid curve portrays the transmittancy of methane after the total pressure of gas within the sample cell was brought to atmospheric conditions by admitting dry air to the cell, the partial pressure of methane remaining unchanged.

In Fig. 8·34 is shown the relationship between the absorbancy and the pressure of methane when only methane is present in the sample cell. An inspection of this figure reveals the fact that the transmittancy of methane does not follow the Beer-Bouguer law. In Figs. 8·35, 8·36, and 8·37 are shown the effects of various diluent gases on the absorb-

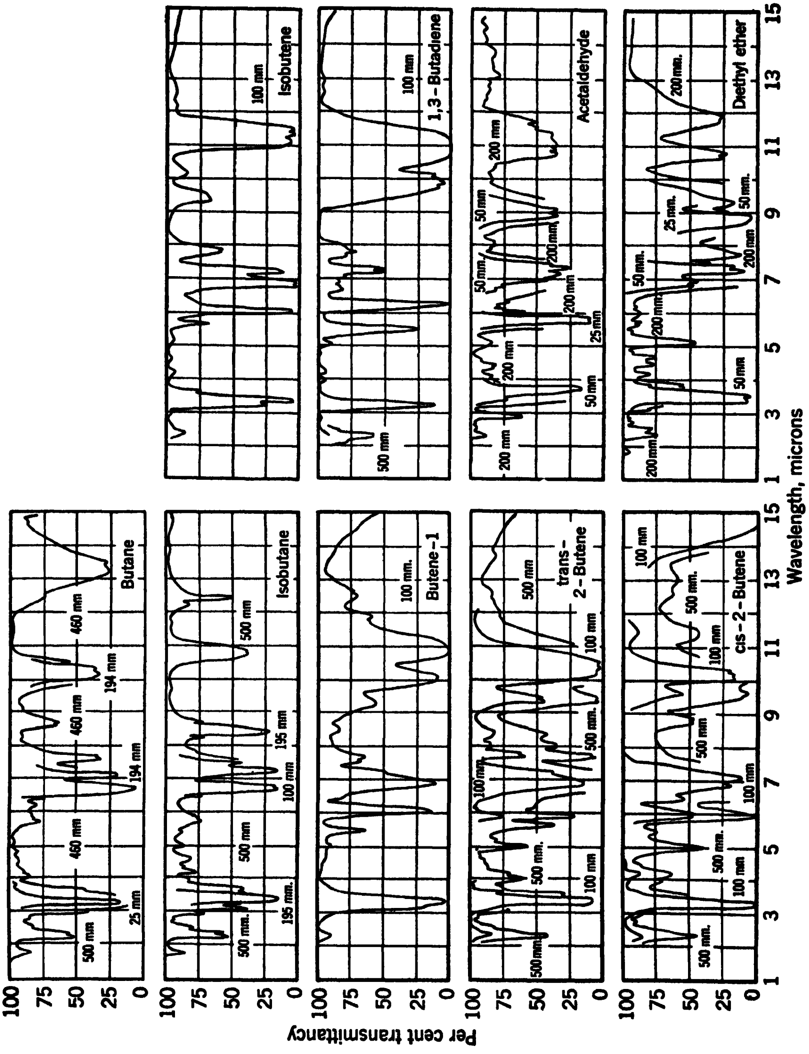


FIG. 8·39. Infrared transmittancy of acetaldehyde, diethyl ether, and the C_4 hydrocarbons.

ancy of the *P*-, *Q*-, and *R*-branches of the 7.58-micron methane band. This information emphasizes the observation that only an empirical method can be established for the analysis of such materials, and, further, that the composition of the sample can vary only within the limits for which calibration data are available.

The effect of various diluent gases on the absorbancies of such materials as CO, CO_2, CH_4, C_2H_2, and C_2H_4 has been discussed extensively in the literature (*1*, *2*, *3*, *4*, *11*, *17*). It is generally agreed that the phenomenon of increasing absorbancy index with decreasing concentration at the same partial pressure is attributable to the "pressure broadening" of spectral lines, caused by collision damping.

It frequently happens in industrial work that the concentration of only one component present in a mixture is of interest. Further, the molecular configuration of the component in question may be considerably different from those of the other materials present. The analysis of refined butadiene presents a problem of this nature (*9*). Refined butadiene contains varying amounts of such impurities as butene-1, butene-2, and perhaps traces of isobutylene and acetylenes, together with small amounts of saturates.

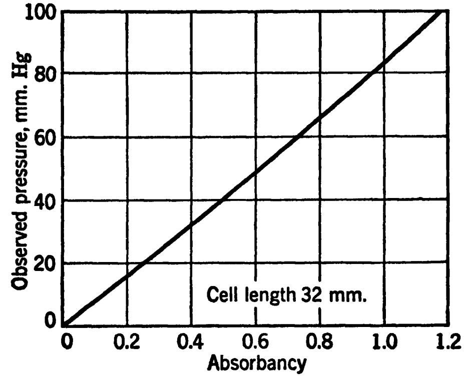


FIG. 8·40. Transmittancy of acetaldehyde at 5.66 μ.

These impurities not only absorb strongly at 6.9 microns, where butadiene is relatively transparent, as shown in Fig. 8·38, but the absorbancies of the major impurities, butene-2 and butene-1, are of the same order of magnitude. As a consequence, it is possible to construct a calibration curve relating the absorbancy of the sample at 6.9 microns with the concentration of impurities present.

Analyses of this type were extended by the author to the determination of the amounts of acetaldehyde and ether present in the C_4 fraction associated with butadiene.

From the transmittancy curves of Fig. 8·39 it can be seen that at 5.66 microns acetaldehyde is the only component of the C_4 cut which absorbs appreciably. At 8.78 microns diethyl ether absorbs strongly, acetaldehyde absorbs moderately, and the C_4 hydrocarbons are nearly transparent

This is shown quantitatively in Figs. 8·40, 8·41, and 8·42. Figure 8·40 shows how the absorbancy of acetaldehyde at 5.66 microns changes with pressure. Similar curves for both acetaldehyde and diethyl ether at 8.78 microns are illustrated in Fig. 8·41. Absorbancy-pressure curves for the C_4 hydrocarbons at 8.78 microns and for diethyl ether as well as the C_4 hydrocarbons at 5.66 microns are shown in Fig. 8·42. An inspection of these figures makes it obvious that the acetaldehyde and diethyl ether content of the C_4 fraction can be calculated from the pres-

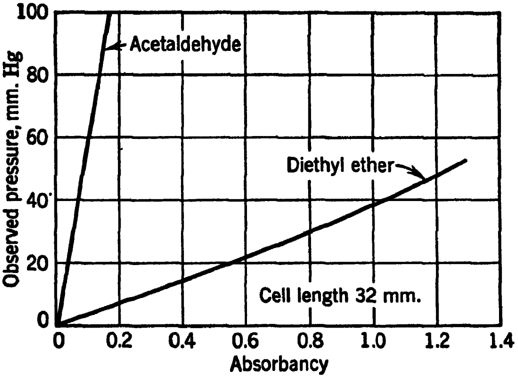


FIG. 8·41. Transmittancy of acetaldehyde and diethyl ether at 8.78 μ.

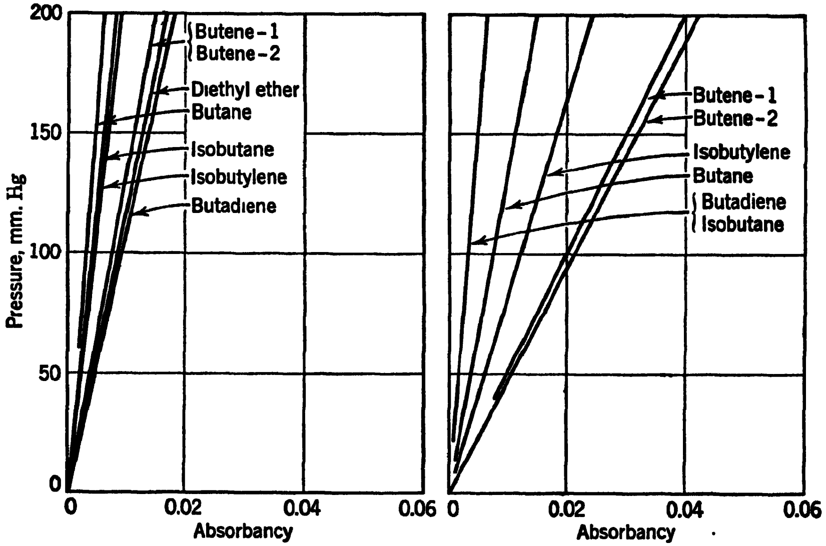


FIG. 8·42. Transmittancy of C_4 hydrocarbons and diethyl ether at 5.66 μ.

sure of tne gaseous sample and its absorbancies at 5.66 and 8.78 microns. It is also evident that the aldehyde content must be evaluated in the process of analyzing for the ether. The converse is also true because of corrections which must be applied to the "observed pressures" of the sample, acetaldehyde, and diethyl ether. These corrections are made so that the concentrations of the aldehyde and ether can be calculated on the basis of the perfect gas law.

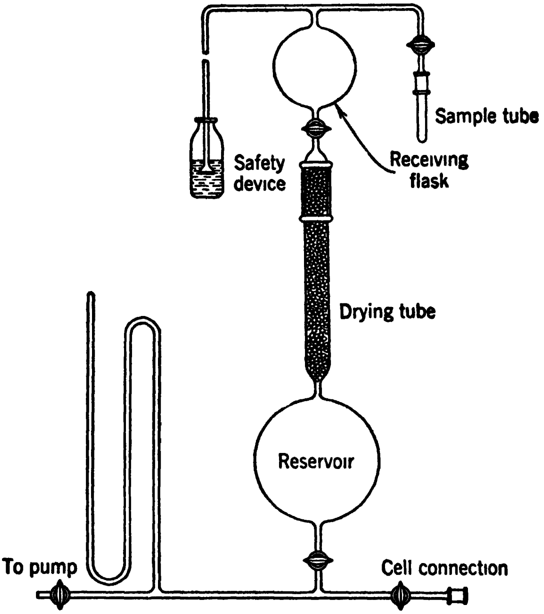


FIG. 8·43. Apparatus for drying and preparing the C_4 fraction containing acetaldehyde and diethyl ether for infrared analysis.

Samples which have been stored for any length of time always contain considerable amounts of water. This can be removed effectively without preferential absorption of the other components by passing the sample over anhydrous $CaCl_2$. The diagram of the apparatus used to dry and prepare the sample is shown in Fig. 8·43. It consists of a sampling tube, 250-ml. receiving flask, $CaCl_2$ tube, and a 1000-ml. reservoir, together with auxiliary equipment such as stopcocks and ground-glass joints, and a manometer for measuring the pressure of the sample.

The sampling tube is chilled in an acetone-dry ice mixture just before the introduction of the sample. Sample and tube are cooled for a few minutes in the acetone-dry ice mixture before connecting the sampling tube to the apparatus. Air is removed from the sample by evacuation, taking care to keep the sample cold. The stopcock between the $CaCl_2$ tube and the receiving flask is then turned off and the sample flashed. The size of the sample is controlled so that the resultant pressure in the receiving flask never exceeds atmospheric. A safety device shown in the diagram is incorporated in the instrument so that dangerous pressures cannot build up. It consists of a fine sintered glass disc connected to the mixing flask. The disc is immersed in mercury. Gas can readily pass through the disc and mercury, but mercury cannot pass through the disc.

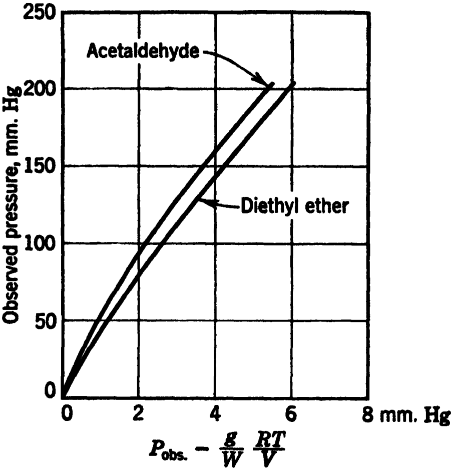


Fig. 8·44. Deviation from ideal gas law at 25°C. for acetaldehyde and diethyl ether.

A warm water bath may be used to heat the sampling tube to room temperature or above, after which the stopcock between the receiving flask and the $CaCl_2$ tube is opened. The desired pressure of approximately 100 mm. Hg is obtained with the vacuum pump, and the sample introduced into a 32-mm. absorption cell in the conventional manner. It is important to renew the $CaCl_2$ frequently in order to secure reliable results.

Obtaining the "observed pressures" of acetaldehyde and diethyl ether from the absorbancy measurements is straightforward. No corrections are usually applied to the absorbancies because of the contributions of the C_4 components, but corrections must be applied to correct for the deviations from the perfect gas law. The corrections for acetaldehyde and diethyl ether at 25°C. are shown in Fig. 8·44. A correction of 0.4 mm. Hg should be added to the observed pressure of the C_4 hydrocarbons when their pressure is 100 mm. Hg.

TABLE 8·7 A COMPARISON OF THE VOLUMETRIC AND INFRARED ABSORPTIMETRIC ANALYSES OF THE C_4 CUT FOR ACETALDEHYDE AND DIETHYL ETHER

	Infrared Analysis		*Volumetric Analysis*	
Sample	% Acetaldehyde	% Diethyl Ether	% Acetaldehyde	% Diethyl Ether
E-152	35.0	0.0	35.4	....
	35.0	0.0	....	....
E-141	42.8	0.0	42.3	....
(73–96)	43.1	0.0	....	....
E-158	22.6	2.2	22.8	....
(cut 3)	22.7	2.2	....	....
	22.5	2.1	....	....
E-142	24.9	0.0	25.3	....
(97–120)	25.6	0.0	....	....
E-143	45.1	0.0	44.8	....
(B)	45.0	0.0	....	....
E-165	32.0	0.7	31.9	....
	....	....	....	....
E-145	35.4	0.9	35.0	....
	35.1	0.9	....	....
E-162	16.3	1.2	16.3	....
	16.3	1.4	....	....
E-169	18.5	0.0	18.1	....
	18.1	0.0	....	....
E-166	10.7	0.0	10.6	....
	10.8	0.0	....	....
E-153	2.5	0.4	2.5	....
	2.5	0.4	....	....
E-179	19.7	0.8	19.6	....
	20.0	0.8	....	....
E-155	19.5	0.5	19.4	....
	19.5	0.3	....	....
E-176	10.2	0.5	10.5	....
	10.4	0.4	....	....
E-174	12.2	0.9	11.8	....
	12.2	0.9	....	....
E-186	10.2	....	10.0	....
	10.2	....	....	....
E-187	10.3	0.2	10.1	....

A comparison of the analytical results obtained by infrared and volumetric methods for acetaldehyde is made in Table 8·7. The diethyl ether content (infrared) is also shown.

Duplicate determinations of the acetaldehyde and diethyl ether content of the C_4 fraction can be made in less than 30 minutes, including all calculations. This method obviously will not differentiate between diethyl and methylethyl or other ethers, and the effect of the latter on the analysis has not been determined.

8·27 Multicomponent gas analyses. The analysis of multicomponent mixtures involves an extension of the principles involved in one- and two-component determinations. This implies that the same number of spectral positions as there are components must be chosen. Further, working curves showing the relation between absorbancy and pressure must be determined for all components at each spectral position. This means that for n components, $n \times n$ or n^2 working curves are necessary.

From these curves the corrections for false energy may be determined as follows. It was shown in equation (8·82) that

$$(A_s)_c = \log_{10} \frac{I_0 - I_f}{I - I_f} \qquad (8\cdot 82)$$

where $(A_s)_c$ is the corrected absorbancy, I_0 is the energy measured by the spectrometer with the sample cell evacuated, I is the energy measured with the gas in the sample cell, and I_f is the false energy correction. I_f is determined by substituting several values of I_f into the above equation until the ratio A_s/p (where p is the corrected sample pressure) is constant up to an absorbancy of 0.6 to 0.7.

From the corrected absorbancies, absorbancy indexes are determined which for convenience are calculated for a 10-cm. corrected sample pressure and a 10-cm. cell, using the following expression:

$$(a_s)_c = (A_s)_c \times \frac{100}{P_c} \times F \qquad (8\cdot 83)$$

Here $(a_s)_c$ is the corrected absorbancy index, $(A_s)_c$ is the corrected absorbancy, P_c is the corrected cell pressure, and F is the temperature correction factor.

In equation (8·78) it was pointed out that the absorbancy A_s of a sample at any wavelength is equal to the sum of the absorbancies of its components. That is,

$$(A_s)_m = (A_s)_1 + (A_s)_2 + (A_s)_3 + \cdots (A_s)_n \qquad (8\cdot 84)$$

where $(A_s)_m$ is the absorbancy of the mixture, and $(A_s)_n$ is the absorbancy of the nth component. This expression may be developed to include absorbancy indexes, so that at any wavelength λ_1

$$(A_s)_{m\lambda_1} = (a_s)_1c_1 + (a_s)_2c_2 + (a_s)_3c_3 + \cdots (a_s)_nc_n \qquad (8\cdot85)$$

where $(A_s)_{m\lambda_1}$ is the corrected absorbancy as evaluated using equation (8·85), $(a_s)_n$ is the corrected absorbancy index, and c_n is the molar concentration of the nth component. In general, n such equations, one for each of the n selected spectral positions, are required in order that the concentration of the individual components can be determined.* That is, in order that c_n can be evaluated, equations must be solved of the form

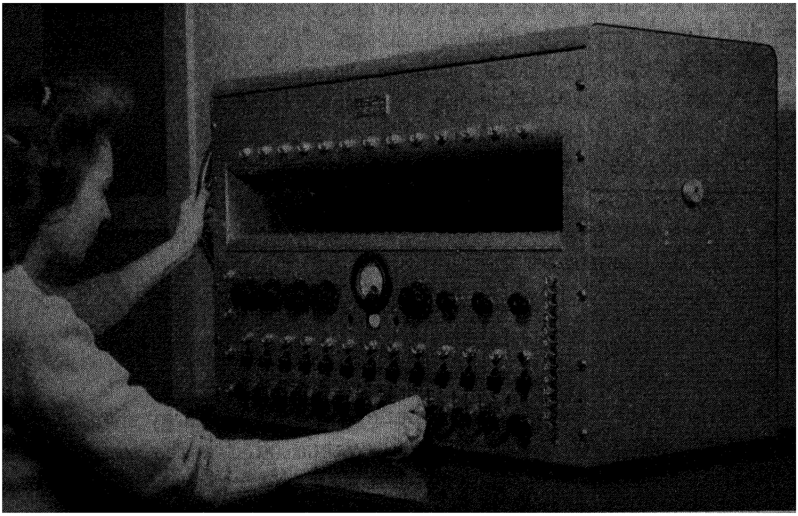


FIG. 8·45. Electrical calculator. (Courtesy of Consolidated Engineering Corporation.)

$$\begin{array}{lcl}
(A_s)_1 & = & (a_s)_1c_1 + (a_s)_2c_2 + (a_s)_3c_3 + \cdots (a_s)_nc_n \\
(A_s)_1{}^1 & = & (a_s)_1{}^1c_1 + (a_s)_2{}^1c_2 + (a_s)_3{}^1c_3 + \cdots (a_s)_n{}^1c_n \\
\cdot & \cdot & \cdot \quad\quad \cdot \quad\quad \cdot \quad\quad \cdot \\
\cdot & \cdot & \cdot \quad\quad \cdot \quad\quad \cdot \quad\quad \cdot \\
\cdot & \cdot & \cdot \quad\quad \cdot \quad\quad \cdot \quad\quad \cdot \\
(A_s)_1{}^{n1} & = & (a_s)_1{}^{n1}c_1 + (a_s)_2{}^{n1}c_2 + (a_s)_3{}^{n1}c_3 + \cdots (a_s)_n{}^{n1}c_n
\end{array} \qquad (8\cdot86)$$

---

* Solution of the simultaneous equations for derivation of the explicit equations is made by any one of several methods discussed in the literature. See, for example, P. D. Crout, *Trans. Am. Inst. Elec. Engrs.*, **60**, 1235 (1941).

Here $(A_s)_1^{n-}$ is the corrected absorbancy of the mixture at the nth spectral position and $(a_s)_n^{n1}$ is the corrected absorbancy index of the nth component at the nth spectral position. A general explicit equation for one component will have the form

$$c_n = \mathrm{A}_n(a_s)_1 + \mathrm{B}_n(a_s)_2 + \Gamma_n(a_s)_3 + \cdots \mathrm{N}_n(a_s)_n \qquad (8\cdot 87)$$

where A_n, B_n, Γ_n, $\cdots$ N_n are factors derived from the solution of equation (8·87).

The present peak of spectrophotometric calculative gadgetry appears in the electrical devices now commercially available for solving the kind of equations involved in multicomponent determinations. Figure 8·45 illustrates one of these calculators.

8·28 Precautions to be taken in gas analyses. 1. The samples used for calibration must be of the highest purity obtainable, that is, 99.5 per cent or better, if possible. If such purity is not obtainable, and the impurities are known to be other gases involved in the analysis, corrections can be applied provided a good analysis of the sample is known. Particular care must be taken that slight amounts of impurities are not present which have relatively much greater absorption than any of the components in the mixture.

2. If there is much difference between the boiling points of the components of any mixture, some trouble may be encountered in obtaining proper mixing of the sample after freezing it in liquid nitrogen. The most satisfactory method of mixing such samples is to confine them in an essentially spherical volume, in which case mixing takes only a few minutes.

8·29 The analyses of liquids. Since liquids are considerably more dense than gases, their refractive indices are higher, and it is therefore no longer possible to determine I_0 for a given sample by measuring the intensity of the energy passing through the empty cell. The reason lies in the fact that more energy is lost by reflection at the two inner surfaces of the cell when empty than when filled with a liquid. This trouble is easily avoided in regions of the spectrum where there are completely transparent liquids which can be used for solvents. In this case the true I_0 and I readings are easily determined, using matched absorption cells filled with the solvent and solution respectively. This technique can be used in almost all cases in the near ultraviolet and visible regions of the spectrum where water and many hydrocarbons are completely transparent over wide ranges of wavelength. In the infrared portion of the spectrum, however, there are no solvents that are completely transparent over even moderate wavelength intervals. This does not completely exclude the use of solvents in problems of analysis, but it definitely limits the number of problems in which they find application.

Where a completely transparent solvent cannot be found, it is necessary to determine a "cell constant," which can be described as the factor which, multiplied by the intensity of energy incident upon the cell, E_0, gives the true I_0, that is,

$$I_0 = \alpha E$$

It can also be described as the fraction of the energy which would be transmitted by the cell if filled with a transparent solvent. The "cell constant" depends on wavelength and changes slowly with time, so that periodic checks of the cell are required. The cell constants for various wavelengths are readily determined by measuring the transmittance of a liquid whose transmission characteristics have been previously determined, using a variable thickness cell (*23*).

8·30 Calibration involving liquids. The methods described for the analysis of gases can be extended to the analysis of liquid mixtures. The data required are the same, both involving the determination of a set of curves representing absorbancy plotted against some function of the concentration for each component at each of the spectral positions. Only the method of determining the data is different.

The type of liquid analysis which is most analogous to that of gases is one in which the analysis is run on the sample dissolved in an inert solvent. Here, instead of determining absorbancies of each gas at a series of pressures, absorbancies of a series of solutions of the pure components are found. Likewise, as was the case with gases, it is possible to base the calibration on a standard concentration for each principal absorber or to adjust the concentration used for an unknown sample of any wavelength until its absorbancy falls between certain limits, usually 0.2 to 0.8. Although calculations vary, depending on the method used in setting up the final analysis, the calibration data necessary for the two are the same; absorbancies are measured on a series of accurately prepared solutions. The range of concentrations to be used should extend so that the highest concentration of each component at each wavelength slightly exceeds the maximum concentration of that component which will ever be encountered in actual analysis.

Where solvents having some absorption must be used, the "cell constant" must be determined. It can be expressed in terms of absorbancy so as to include the absorbance of the solvent, thus including corrections for the losses due to the cell as well as those due to the absorption of the solvent.

Liquid analyses which involve analyses where the liquid samples must be run undiluted require that the calibration be determined for binary mixtures of the components which cover the concentration ranges to be encountered in the actual analysis. The concentration can be expressed in any way so that it represents the number of molecules per milliliter of sample. If the molecular weights of all components are the same, percentage by weight is satisfactory; otherwise percentage by volume may have to be used.

At any wavelength, the component which absorbs the least is assumed to follow the Beer-Bouguer law exactly, that is, that its absorbancy-concentration curve is a straight line. All other curves at this wavelength are then referred to this straight line, which is drawn from the absorbancy of the pure component on the 100 per cent axis to the origin. In this case, "cell constants" must always be determined and used in calculation of the absorbancies of the liquid mixtures. The calculated absorbancy of the minor absorber (determined with the assumption that it follows the Beer-Bouguer law) can then be subtracted from that of the mixture to get the absorbancy of the major absorber to be used in plotting the usual calibration curves.

8·31 The "base-line" technique. In 1941 Wright (*60*) described a "base-line" procedure for the analysis of liquid organic compounds. The importance of this technique was realized immediately, and as a consequence it was developed until the concentration of as many as nine paraffinic hydrocarbons in a mixture can now be determined, using this method (*28*). The "base-line" procedure, when compared to the conventional methods, has the following advantages: it is faster, the calibration steps are easier to carry out, and the calculations are simplified. In addition, it can be extended to the analysis of difficultly soluble solids since such materials can be mulled with Nujol, placed on a rock-salt plate, and their absorption curves determined. From these curves the concentration of impurities can be evaluated. This is illustrated in developing a method for the determination of the amount of ethionine in methionine. In general, such analyses involve (1) a qualitative determination of the compounds present by inspection of the complete spectrum, (2) measurement of the base-line absorbancies at the selected spectral positions, (3) substitution of these values in the appropriate equations, and (4) solution of the equations to obtain the concentration of the components.

The procedure employed consists of obtaining the spectrum of the pure compounds superimposed on the radiant energy background. This information is used to establish the most nearly unique absorption peak for each compound (*43*). As scanning toward longer wavelength progresses, the amount of radiant energy reaching the thermocouple decreases. Therefore, it is necessary to increase the slit widths whenever the recorded spectrum falls to 40 per cent of full chart reading. This results in producing absorption curves with a saw-tooth effect.

The absorption peaks chosen for analysis are made from a detailed consideration of the spectra of all components and are chosen so as to minimize interference between two or more compounds. The intensity of absorption of the individual peaks is expressed in terms of "base-line" absorbancy as

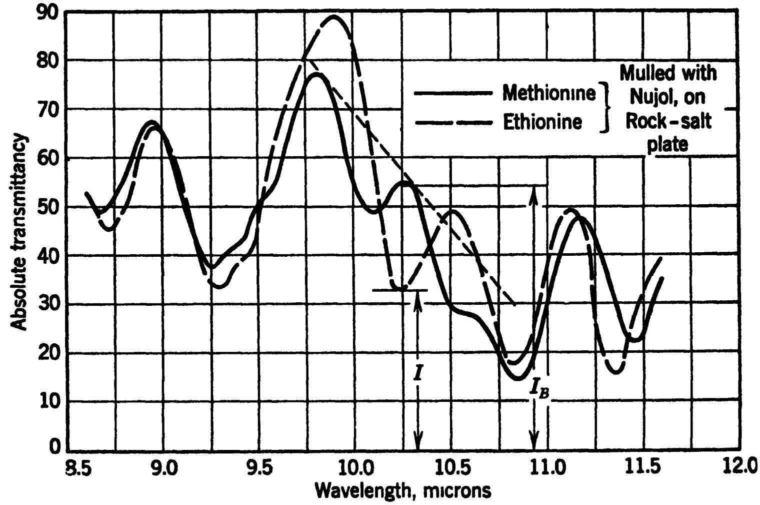


FIG. 8·46. The determination of I and I_B for "base-line" analysis.

$$(A_s)_B = \log_{10} \frac{I_B}{I} \tag{8·88}$$

where $(A_s)_B$ is the "base-line" absorbancy, I is the distance on the recorded spectrum from the zero line to the selected absorption peak, and I_B is the distance from the zero line to a straight line, the base-line, joining two spectral points located near the absorption peak. I_B and I are both measured at the same wavelength. The method of determining I and I_B is illustrated in Fig. 8·46.

The values obtained from the base-line absorbancies of all the absorption peaks are used to develop equations similar to equations (8·86) and (8·87). That is:

$$
\begin{array}{llllll}
(A_s)_B &= (a_s)_1c_1 &+ (a_s)_2c_2 &+ (a_s)_3c_3 &+\cdots+ (a_s)_nc_n & \\
(A_s)_B{}^1 &= (a_s)_1{}^1c_1 &+ (a_s)_2{}^1c_2 &+ (a_s)_3{}^1c_3 &+\cdots+ (a_s)_n{}^1c_n & \\
\cdot & \cdot & \cdot & \cdot & \cdot \qquad \cdot & \qquad (8\cdot 89) \\
\cdot & \cdot & \cdot & \cdot & \cdot \qquad \cdot & \\
\cdot & \cdot & \cdot & \cdot & \cdot \qquad \cdot & \\
(A_s)_B{}^{n1} &= (a_s)_1{}^{n1}c_1 &+ (a_s)_2{}^{n1}c_2 &+ (a_s)_3{}^{n1}c_3 &+\cdots+ (a_s)_n{}^{n1}c_n &
\end{array}
$$

and

$$c_B = \mathrm{A}_n(a_s)_1 + \mathrm{B}_n(a_s)_2 + \Gamma_n(a_s)_3 + \cdots + \mathrm{N}_n(a_s)_n \qquad (8\cdot 90)$$

8·32 Other applications. Current developments in infrared spectrometry are taking place much more rapidly than those for the ultraviolet and visible regions. This applies both to instruments and to applications.

Nearly every issue of the relevant journals carry new items. To be reasonably up to date, therefore, the analyst must be alert to the work published in such sources. It is one more example of the uncertainty always prevailing with any kind of method until instrumental developments reach some degree of stability and until possible kinds of applications have undergone the usual survey stage. [See review by V. Z. Williams in *Rev. Sci. Instruments*, **19**, 135–178 (1948).]

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9· MEASUREMENT AND SPECIFICATION OF COLOR *

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A·GENERAL CONSIDERATIONS

9·1 Introduction. It is common practice to regard color as a property of objects, and in a limited sense this is justified. We have color comparators for solutions in which the color is taken as an index of the composition of the solution; and in applying a suitably prepared set of color standards in a color comparator, the color of the unknown behaves as if it were a property of the solution itself, just as truly as the concentrations of the constituents which it indicates. However, this chapter deals with color for its own sake; and, for this purpose, a broader view is useful. Lights have colors as well as objects. The flame of a Bunsen burner can be changed from bluish purple to orange by the introduction of sodium. And since even objects lose their colors and become invisible unless they reflect, scatter, or transmit radiant energy, or form a part of an illuminated scene, this broader view is that color is a property of light, and of light alone.

Since it is possible to measure by a spectrophotometer the spectral energy distribution of any light beam, and since the color of a light correlates closely with its spectral composition, some of the more physically minded have contended that color is a physical property of radiant energy; but this is not the most useful view. The color change of the Bunsen flame from bluish purple to orange can be shown by a purely physical measurement to be caused by a change in the spectral composition of the emitted energy, but it takes more than physics to decide whether this flame has the same color as the light reflected from the peel of a given citrus fruit. Application of the spectrophotometer to the orange peel will show that the spectral composition of the light reflected from it under daylight illumination is radically different from that emitted by the sodium flame. It has a continuous spectrum relatively strong in the long-wave portion of the visible spectrum (380 to 770 mμ). The visible energy of the sodium flame is nearly all confined to two narrow bands (589.0 and 589.6 mμ). Physically, therefore, the two lights are different, but they have closely the same color. The two lights must therefore be identical in some other respect. This identity consists in some aspect of the response made by a normal observer to the sodium flame being the same as the corresponding aspect of the response to the peel of the citrus fruit. The broader view of color must, therefore, include not only the spectral composition of the radiant energy reaching the eye of the observer, but the properties of the observer as well. These properties have been evaluated by finding equivalent stimuli, like the energy of the sodium flame and that reflected from an orange peel, which have different spectral compositions but still manage to have the same color to the normal observer. Such equivalent stimuli are called *metamers*. In this chapter there will be presented the standard method for finding by computation whether or not any two lights form equivalent stimuli. The fundamental method of color specification based upon equivalent stimuli plus spectrophotometry will be described in detail together with other methods of obtaining the same numbers. And finally some discussion will be given of the use of the Munsell color system and the I.S.C.C.-N.B.S. method of color designation in accord with *American War Standard for the Specification and Description of Color* Z44-1942 (*5*).

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* See Division F for definitions of various color terms.

9·2 Definition of color. The most widely accepted technical definition of color is that given by the Committee on Colorimetry of the Optical Society of America (*15*): "Color consists of the characteristics of light other than spatial and temporal inhomogeneities; light being that aspect of radiant energy of which a human observer is aware through the visual sensations which arise from the stimulation of the retina of the eye." It will be noted that this definition relates color and light to radiant energy only in so far as the energy produces a visual effect within an observer. On this account color and light are said to be psychophysical entities, and in their evaluation it is ordinarily not necessary to pay attention to energy of wavelength less than 380 mμ nor greater than 770 mμ because the eye is relatively insensitive to such energy.

9·3 Tridimensionality of color. If a normal observer attempts to adjust one element of his visual field whose color is under his control so that it matches a neighboring element, he will ultimately discover that three independent adjustments have to be at his disposal. If he is using the red, yellow, and blue paints frequently found in primary grade schools, only by chance will he obtain a match from a mixture of two of them. Even a brown color requires blue in addition to red and yellow. Within the color gamut of the three paints, an exact match for any given color is easily possible, but three is the irreducible minimum. Similarly, if he is trying to color-match one spot of light by shining several spotlights of different colors onto the same neighboring spot of a screen, he finds, in general, that either three lights of fixed spectral composition are required, or, if only two lights be added together, not only the amounts of both but also the spectral composition of at least one has to be adjustable. The same rule applies to rotary mixture on a sector disc; four sectors, giving three independent adjustments, are necessary and sufficient.

Since the color vision of a normal observer is tridimensional, it follows that there must be at least three independent excitations in the optic-nerve fibers corresponding to each patch of the visual field. Theories of color vision consist of speculation as to the character of these excitations. It also follows that a color specification is expressible by three numbers. For normal observers three numbers are necessary; for partially color-blind observers only two numbers are necessary; and for totally color-blind observers only one is necessary.

In the examples given (paints, spotlights, sector discs) the observer by adjustment of three variables obtains a color match, that is, he has to set up a second stimulus equivalent to the first. Except by accident, however, the ternary or binary mixture does not match the unknown in spectral composition. In the usual case the mixture is equivalent to the unknown in color but not in spectral composition, and the unknown and the mixture are said to form a *metameric pair*. There are, however, degrees of difference in spectral composition. If one painted panel be matched by a mixture of red, yellow, and blue paints, the degree of metamerism is likely to be only moderate; but if the paint panel illuminated by daylight be matched by shining on a white card three spotlights each of which contains energy restricted to a narrow wavelength band (such as spectrum red, green, and blue), the degree of metamerism will ordinarily be large.

9·4 Metamerism and the macular pigment. Studies of extremely metameric pairs in which mixtures of two parts of the spectrum are set up to color-match other two-part spectrum mixtures have yielded our most valuable knowledge regarding the properties of the average normal eye (*1*, *32*, *55*, *66*, *112*). An outstanding fact derived from observation of such metamers is that the center of the retina (fovea centralis) has somewhat different properties from that part of the retina immediately surrounding it; that is, a color match set up for the central 2 or 3 degrees of the retina becomes an easily detectable mismatch if the eye be turned so as to allow the stimuli forming the metameric pair to affect a portion of the retinas, say, 6 degrees from the fovea. Furthermore, if the metamers are compared in large patches so as to subtend 6 degrees or more at the eye of the observer, this mismatch causes a central spot to appear temporarily on a field that is physically uniform. This dependence of metamerism on the portion of the retina used arises chiefly from the existence of a spot of brownish or yellowish pigment irregularly covering and interpenetrating the central 3 or 4 degrees of the normal retina; it is called the *macula lutea* or sometimes the *yellow spot* (see Fig. 9·1). Figure 9·1 shows a horizontal cross-section of the eye. Light enters the tear-film *ff*, passes through the cornea *aa*, the aqueous humor *B*, the pupil *bb*, the crystalline lens *A*, the vitreous humor *C*, and the macula *p* before reaching the retina *i*. The macular pigment acts as a selective filter interposed between the vitreous humor *C* and the retina *i*. Metamers set up for one normal observer usually fail to hold strictly for anyone else. This failure is ascribable to variations in amount of pigmentation of the eye media (cornea, lens, humors, macula), the macular pigment being one of the chief variables. The properties of the normal eye derived from a study of these extreme metamers therefore refer only to the central 2 degrees of the retina, and they refer to an hypothetical average eye. Nobody has been found

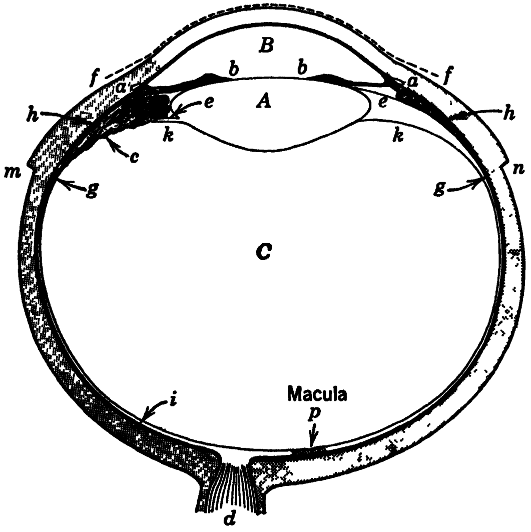

Fig. 9·1. Horizontal cross-section of the normal human eye. (After Helmholtz.) Note that the macular pigment acts as a yellow filter interposed between vitreous humor *C* and retina *i*.

whose eye differs so little from this average eye that the differences could not be detected. Practically speaking, therefore, nobody has an eye that is colorimetrically normal.

9·5 Grassman's law. From a knowledge of spectral metamers it has been possible to summarize concisely the properties of the average normal eye. This summary is made in accord with the principle known as Grassman's law (*29*) foreshadowed by Newton's laws of color mixture. If a light composed of known amounts of three components (called primaries) is equivalent in color to an unknown light, the three known amounts may be used as a color specification for this light. These amounts are called the *tristimulus values* of the color. Grassman's law states that, when equivalent lights are added to equivalent lights, the sums are equivalent. Thus, if an unknown spot of color were matched by shining on the same spot of a white screen two component spotlights of tristimulus values, X_1, Y_1, Z_1, and X_2, Y_2, Z_2, respectively, by Grassman's law, the tristimulus values, X, Y, Z, of the unknown spot of color would be simply:

$$\begin{aligned} X &= X_1 + X_2 \\ Y &= Y_1 + Y_2 \\ Z &= Z_1 + Z_2 \end{aligned} \tag{9·1}$$

Any beam of light, whether it originates from a self-luminous body or comes, by transmission, scattering, or reflection, from a non-self-luminous object, may be considered as made up of a large number of portions of the spectrum. The amounts of these various portions may be determined by spectrophotometry. The tristimulus specifications, $\bar{x}_\lambda$, $\bar{y}_\lambda$, $\bar{z}_\lambda$, of each of these portions have been determined for a number of normal observers, and average values are given in Table 9·1 in arbitrary units for a spectrum of unit spectral irradiance.*

The principle expressed in Grassman's law has been established by repeated experiment over a wide middle range of retinal illuminations. It breaks down for very high retinal illuminations (*113*) that begin to approach those sufficient to do the eye permanent harm, and it breaks down if the illumination of the whole retina continues for several minutes to be so slight that vision by the retinal rods (twilight vision) intrudes significantly (*56*). Between these two extremes, however, Grassman's law holds independently of the adaptive state of the eye. Thus, if two stimuli of different wavelength distributions of energy be found that are once responded to alike by the eye, they will be seen alike even after exposure of the eye to another stimulus sufficient to

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* See Division F, Definitions.

TABLE 9·1 THE 1931 I.C.I. STANDARD OBSERVER

Wave-length, mμ	Tristimulus Specifications of Equal-energy Spectrum			Wave-length, mμ	Tristimulus Specifications of Equal-energy Spectrum		
	$\bar{x}$	$\bar{y}$	$\bar{z}$		$\bar{x}$	$\bar{y}$	$\bar{z}$
380	0.0014	0.0000	0.0065	580	0.9163	0.8700	0.0017
385	.0022	0.0001	0.0105	585	0.9786	.8163	.0014
390	.0042	0.0001	0.0201	590	1.0263	.7570	.0011
395	.0076	0.0002	0.0362	595	1.0567	.6949	.0010
400	.0143	0.0004	0.0679	600	1.0622	.6310	.0008
405	.0232	0.0006	0.1102	605	1.0456	.5668	.0006
410	.0435	0.0012	0.2074	610	1.0026	.5030	.0003
415	.0776	0.0022	0.3713	615	0.9384	.4412	.0002
420	.1344	0.0040	0.6456	620	0.8544	.3810	.0002
425	.2148	0.0073	1.0391	625	0.7514	.3210	.0001
430	.2839	0.0116	1.3856	630	0.6424	.2650	.0000
435	.3285	0.0168	1.6230	635	0.5419	.2170	.0000
440	.3483	0.0230	1.7471	640	0.4479	.1750	.0000
445	.3481	0.0298	1.7826	645	0.3608	.1382	.0000
450	.3362	0.0380	1.7721	650	0.2835	.1070	.0000
455	.3187	0.0480	1.7441	655	0.2187	.0816	.0000
460	.2908	0.0600	1.6692	660	0.1649	.0610	.0000
465	.2511	0.0739	1.5281	665	0.1212	.0446	.0000
470	.1954	0.0910	1.2876	670	0.0874	.0320	.0000
475	.1421	0.1126	1.0419	675	0.0636	.0232	.0000
480	.0956	0.1390	0.8130	680	0.0468	.0170	.0000
485	.0580	0.1693	0.6162	685	0.0329	.0119	.0000
490	.0320	0.2080	0.4652	690	0.0227	.0082	.0000
495	.0147	0.2586	0.3533	695	0.0158	.0057	.0000
500	.0049	0.3230	0.2720	700	0.0114	.0041	.0000
505	.0024	0.4073	0.2123	705	0.0081	.0029	.0000
510	.0093	0.5030	0.1582	710	0.0058	.0021	.0000
515	.0291	0.6082	0.1117	715	0.0041	.0015	.0000
520	.0633	0.7100	0.0782	720	0.0029	.0010	.0000
525	.1096	0.7932	0.0573	725	0.0020	.0007	.0000
530	.1655	0.8620	0.0422	730	0.0014	.0005	.0000
535	.2257	0.9149	0.0298	735	0.0010	.0004	.0000
540	.2904	0.9540	0.0203	740	0.0007	.0003	.0000
545	.3597	0.9803	0.0134	745	0.0005	.0002	.0000
550	.4334	0.9950	0.0087	750	0.0003	.0001	.0000
555	.5121	1.0002	0.0057	755	0.0002	.0001	.0000
560	.5945	0.9950	0.0039	760	0.0002	.0001	.0000
565	.6784	0.9786	0.0027	765	0.0001	.0000	.0000
570	.7621	0.9520	0.0021	770	0.0001	.0000	.0000
575	.8425	0.9154	0.0018	775	0.0000	.0000	.0000
580	.9163	0.8700	0.0017	780	0.0000	.0000	.0000
				Totals	21.3713	21.3714	21.3715

change considerably the appearance of the two equivalent stimuli. For example, if a portion of the spectrum near 640 mμ (red) be superposed on a portion near 550 mμ (yellowish green), it will be found possible to obtain the color of this combination from an intermediate portion of the spectrum, say, 590 mμ (orange). If the retina of the eye be highly illuminated by light of wavelength near 640 mμ, and its sensitivity to radiant flux of this wavelength region considerably reduced in this way, it is found that, although neither of the equivalent stimuli any longer appears orange, they still give identical colors; for example, they may yield identical yellows or identical greenish yellows. The eye thus cannot be trusted to yield the same color perception from a given stimulus; simultaneous and successive contrast affect it profoundly. But it is still a satisfactory null instrument and obeys Grassman's law.

By Grassman's law it is possible to test whether any two beams of light of differing spectral composition form a metameric pair. The condition for metamerism of two beams of light of spectral irradiance, E_1 and E_2, is that simultaneously:

$$\begin{aligned}
\sum_0^\infty (E_1)_\lambda \bar{x}_\lambda \,\Delta\lambda &= \sum_0^\infty (E_2)_\lambda \bar{x}_\lambda \,\Delta\lambda \\
\sum_0^\infty (E_1)_\lambda \bar{y}_\lambda \,\Delta\lambda &= \sum_0^\infty (E_2)_\lambda \bar{y}_\lambda \,\Delta\lambda \qquad (9\cdot 2) \\
\sum_0^\infty (E_1)_\lambda \bar{z}_\lambda \,\Delta\lambda &= \sum_0^\infty (E_2)_\lambda \bar{z}_\lambda \,\Delta\lambda
\end{aligned}$$

The wavelength interval, $\Delta\lambda$, to be used in these summations depends upon the irregularity of the curve of spectral irradiance with wavelength; intervals of 20 mμ are sometimes sufficient to yield a significant result; intervals of 10 mμ often do; and intervals of 5 mμ are usually sufficient except for discontinuous spectral distributions such as those characterizing gaseous discharge lamps.

The summations of equation (9·2) form the tristimulus values of the color and are customarily given the symbols X, Y, Z, so that the condition for a color match would ordinarily be written:

$$\begin{aligned}
X_1 &= X_2 \\
Y_1 &= Y_2 \qquad (9\cdot 2a) \\
Z_1 &= Z_2
\end{aligned}$$

and would mean that the two colors are identical since their tristimulus values are identical. Thus, to match color number one requires X_1

parts of the X primary, Y_1 parts of the Y primary, and Z_1 parts of the Z primary; and since to match color number two requires the same amounts of the same primaries, the two colors, one and two, are the same.

Any three lights may be used as primaries in a system of tristimulus color specifications, provided only that no one of them is equivalent to a combination of the other two. Tristimulus specifications X, Y, Z, expressed relative to one set of primaries, may be transformed into specifications R, G, B, relative to any other set, by transformation equations of the form:

$$\begin{aligned} R &= K_1X + K_2Y + K_3Z \\ G &= K_4X + K_5Y + K_6Z \\ B &= K_7X + K_8Y + K_9Z \end{aligned} \tag{9·3}$$

The constants K_1 to K_9 may take on any arbitrary values, positive, negative, or zero, provided they are not such as to make one of the new primaries identical to a combination of the other two; that is, provided that:

$$\begin{vmatrix} K_1 & K_2 & K_3 \\ K_4 & K_5 & K_6 \\ K_7 & K_8 & K_9 \end{vmatrix} \neq 0 \tag{9·3a}$$

Since the exceptions that cause the determinant of the system to vanish are trivial, the choice of coordinate system is very wide. The primaries do not even have to correspond to physically realizable lights. Imaginary lights defined by spectral compositions having negative values for some parts of the spectrum are admissible, and indeed are preferred for routine colorimetry because by their use the computation of tristimulus values from spectrophotometric data is somewhat simplified.

B·THE 1931 I.C.I. STANDARD COLORIMETRIC COORDINATE SYSTEM

9·6 General principles. In 1931 the International Commission on Illumination recommended that all subsequent color data be expressed in terms of the same tristimulus system so that the results would be immediately comparable. The standard observer and coordinate system recommended (*36*, *45*, *93*, *102*) is defined by the tristimulus values of the spectrum colors given in Table 9·1 and plotted in Fig. 9·2. It will be noted that the primaries chosen are such that none of these tristimulus values is less than zero. It is further true that the green primary chosen, whose amounts are designated by Y, is such as to carry all of the luminosity, the other two primaries (red, blue) whose amounts are designated by X and Z, respectively, being unassociated with luminosity. Therefore the values of $\bar{y}$ for the spectrum correspond to the standard luminosity function, and it is convenient and customary to express the Y value of a luminous area as its *luminance* (photometric brightness) in terms of some recognized unit (such as candles per square meter, millilambert, or foot-Lambert). The Y value of an opaque specimen may be conveniently expressed as its *luminous reflectance* (ratio of reflected to incident luminous flux); and the Y value of a transmitting specimen is customarily put in terms of *luminous transmittance* (ratio of transmitted to incident luminous flux).

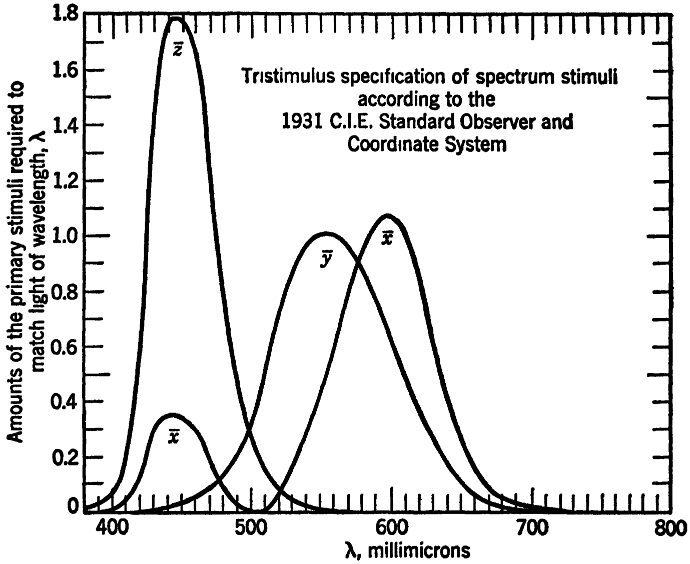


FIG. 9·2. Tristimulus values of the spectrum colors according to the 1931 I.C.I. Standard Observer. (C.I.E. is French abbreviation; see ref. *93*.)

If, as is usual, light combinations are not the chief interest, it is convenient to substitute for the tristimulus values, X, Y, Z, the two ratios, $X/(X + Y + Z)$ and $Y/(X + Y + Z)$, combined with the luminous value, Y. The two ratios are known as *chromaticity coordinates*, x, y, because they serve to specify the chromatic aspect of the light. The analogous ratio, $Z/(X + Y + Z)$, is also known as a chromaticity coordinate, z, but only two of the three coordinates, x, y, z, give independent information since by definition the sum of all three is unity.

TABLE 9·2 CHROMATICITY COORDINATES OF THE SPECTRUM COLORS

Wavelength, mμ	Chromaticity Coordinates			Wavelength, mμ	Chromaticity Coordinates		
	x	y	z		x	y	z
380	0.1741	0.0050	0.8209	580	0.5125	0.4866	0.0009
385	.1740	.0050	.8210	585	.5448	.4544	.0008
390	.1738	.0049	.8213	590	.5752	.4242	.0006
395	.1736	.0049	.8215	595	.6029	.3965	.0006
400	.1733	.0048	.8219	600	.6270	.3725	.0005
405	.1730	.0048	.8222	605	.6482	.3514	.0004
410	.1726	.0048	.8226	610	.6658	.3340	.0002
415	.1721	.0048	.8231	615	.6801	.3197	.0002
420	.1714	.0051	.8235	620	.6915	.3083	.0002
425	.1703	.0058	.8239	625	.7006	.2993	.0001
430	.1689	.0069	.8242	630	.7079	.2920	.0001
435	.1669	.0086	.8245	635	.7140	.2859	.0001
440	.1644	.0109	.8247	640	.7190	.2809	.0001
445	.1611	.0138	.8251	645	.7230	.2770	.0000
450	.1566	.0177	.8257	650	.7260	.2740	.0000
455	.1510	.0227	.8263	655	.7283	.2717	.0000
460	.1440	.0297	.8263	660	.7300	.2700	.0000
465	.1355	.0399	.8246	665	.7311	.2689	.0000
470	.1241	.0578	.8181	670	.7320	.2680	.0000
475	.1096	.0868	.8036	675	.7327	.2673	.0000
480	.0913	.1327	.7760	680	.7334	.2666	.0000
485	.0687	.2007	.7306	685	.7340	.2660	.0000
490	.0454	.2950	.6596	690	.7344	.2656	.0000
495	.0235	.4127	.5638	695	.7346	.2654	.0000
500	.0082	.5384	.4534	700	.7347	.2653	.0000
505	.0039	.6548	.3413	705	.7347	.2653	.0000
510	.0139	.7502	.2359	710	.7347	.2653	.0000
515	.0389	.8120	.1491	715	.7347	.2653	.0000
520	.0743	.8338	.0919	720	.7347	.2653	.0000
525	.1142	.8262	.0596	725	.7347	.2653	.0000
530	.1547	.8059	.0394	730	.7347	.2653	.0000
535	.1929	.7816	.0255	735	.7347	.2653	.0000
540	.2296	.7543	.0161	740	.7347	.2653	.0000
545	.2658	.7243	.0099	745	.7347	.2653	.0000
550	.3016	.6923	.0061	750	.7347	.2653	.0000
555	.3373	.6589	.0038	755	.7347	.2653	.0000
560	.3731	.6245	.0024	760	.7347	.2653	.0000
565	.4087	.5896	.0017	765	.7347	.2653	.0000
570	.4441	.5547	.0012	770	.7347	.2653	.0000
575	.4788	.5202	.0010	775	.7347	.2653	.0000
580	.5125	.4866	.0009	780	.7347	.2653	.0000

Table 9·2 gives the chromaticity coordinates, x, y, z, of the spectrum colors; and Fig. 9·3 shows the points representing the spectrum colors in the (x, y)-chromaticity diagram. This diagram is also known as a *Maxwell triangle* because of Maxwell's first use of such a diagram (*65*).

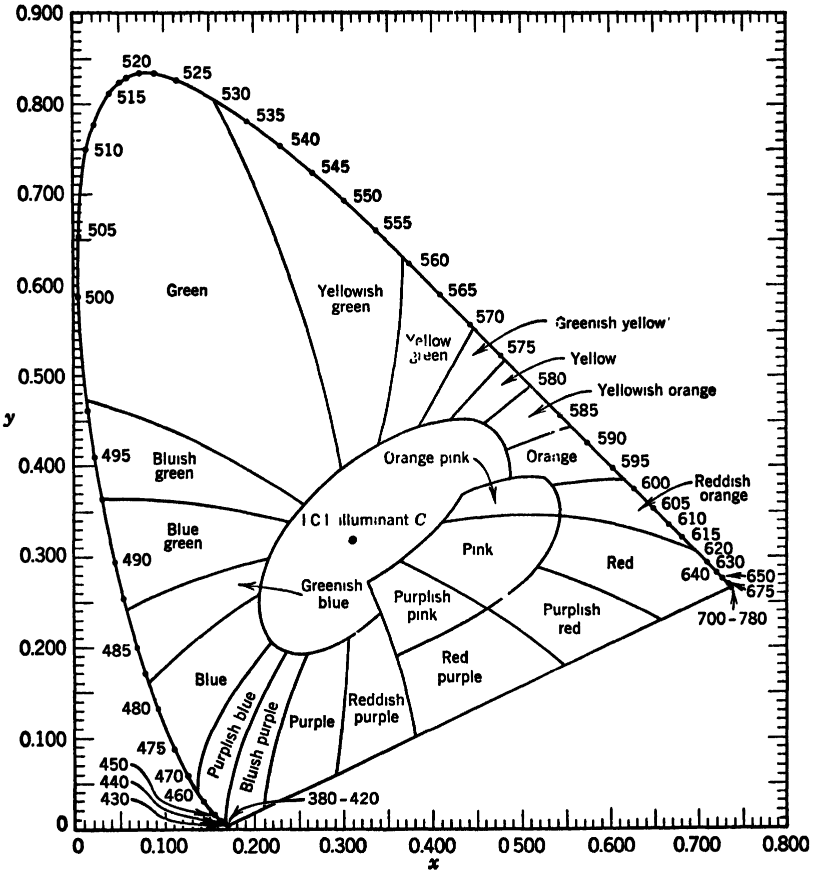


Fig. 9·3. The (x, y)-chromaticity diagram, showing the spectrum locus and the purple boundary. Wavelength is indicated in millimicrons. The hue names are those proposed by Kelly (*52*).

Furthermore, it has aptly been called a *mixture diagram* because it indicates in a very simple way the chromaticity of the color resulting from the additive combination of any two lights. The point representing this chromaticity is found on the straight line connecting the points representing the two lights. The primary lights are represented by points at the corners of a triangle, and every point within the triangle represents the chromaticity of a mixture of the primary lights whose proportions are indicated by the chromaticity coordinates, x, y, z. The spectrum colors are shown by dots connected by a smooth curve known as the spectrum locus. They are identified by wavelength in millimicrons. It will be noted from Fig. 9·3 that the spectrum locus is substantially straight from 540 mμ to the long-wave extreme. This means that the standard observer would find binary mixtures of, say, 540 mμ with 640 mμ, closely equivalent to some intermediate portion of the spectrum. But the spectrum locus from 540 mμ to the short-wave extreme is convex. This means that for the standard observer a binary mixture of 540 mμ with, say, 440 mμ would differ importantly in chromaticity from the intermediate parts of the spectrum. By drawing straight lines through any central point (such as $x = y = \frac{1}{3}$, representing the so-called equal-energy stimulus) and extending them until they cut the spectrum locus, we may find the spectral complementaries relative to a stimulus represented by that point; that is, we may find the two parts of the spectrum that, when combined in proper proportions, will for the standard observer be equivalent to the central stimulus.

The straight line in Fig. 9·3 joining the extremes of the spectrum locus represents the chromaticities of the mixtures of the two extremes of the visible spectrum. The area bounded by the closed curve made up of the spectrum locus and this straight line is the locus of all physically realizable chromaticities. Note that the points representing the primaries of the I.C.I. coordinate system, the apices of the triangle ($x = 1$, $y = z = 0$; $y = 1$, $x = z = 0$; $z = 1$, $x = y = 0$), all fall outside this area; that is, the primaries are imaginary. Note also that both the X and Z primaries fall on the line $y = 0$, which is unassociated with luminosity and is known as the *Alychne* or *lightless line*. The short-wave extreme of the spectrum locus comes close to this line; this means that, although it has the power to elicit in the standard observer a considerable X and Z response, resulting in a vivid bluish purple color, radiant flux of wavelength 380 to 420 mμ is almost unassociated with luminosity.

The areas in Fig. 9·3 corresponding to common color designations for lights are those proposed by Kelly (*52*) and will be discussed later (Division D).

9·7 Standard illuminants. At the time of setting up the standard observer and coordinate system, the International Commission on Illumination (*93*) recommended use of three standard illuminants for colorimetry: illuminant A, representative of gas-filled incandescent lamps; illuminant B, representative of noon sunlight; and illuminant C, TABLE 9·3 RELATIVE SPECTRAL IRRADIANCE OF THE THREE STANDARD ILLUMINANTS, *A*, *B*, AND *C*

Wave-length, mμ	E_A	E_B	E_C	Wave-length, mμ	E_A	E_B	E_C
380	9.79	22.40	33.00	580	114.44	101.00	97.80
385	10.90	26.85	39.92	585	118.08	100.07	95.43
390	12.09	31.30	47.40	590	121.73	99.20	93.20
395	13.36	36.18	55.17	595	125.39	98.44	91.22
400	14.71	41.30	63.30	600	129.04	98.00	89.70
405	16.15	46.62	71.81	605	132.70	98.08	88.83
410	17.68	52.10	80.60	610	136.34	98.50	88.40
415	19.29	57.70	89.53	615	139.99	99.06	88.19
420	21.00	63.20	98.10	620	143.62	99.70	88.10
425	22.79	68.37	105.80	625	147.23	100.36	88.06
430	24.67	73.10	112.40	630	150.83	101.00	88.00
435	26.64	77.31	117.75	635	154.42	101.56	87.86
440	28.70	80.80	121.50	640	157.98	102.20	87.80
445	30.85	83.44	123.45	645	161.51	103.05	87.99
450	33.09	85.40	124.00	650	165.03	103.90	88.20
455	35.41	86.88	123.60	655	168.51	104.59	88.20
460	37.82	88.30	123.10	660	171.96	105.00	87.90
465	40.30	90.08	123.30	665	175.38	105.08	87.22
470	42.87	92.00	123.80	670	178.77	104.90	86.30
475	45.52	93.75	124.09	675	182.12	104.55	85.30
480	48.25	95.20	123.90	680	184.43	103.90	84.00
485	51.04	96.23	122.92	685	188.70	102.84	82.21
490	53.91	96.50	120.70	690	191.93	101.60	80.20
495	56.85	95.71	116.90	695	195.12	100.38	78.24
500	59.86	94.20	112.10	700	198.26	99.10	76.30
505	62.93	92.37	106.98	705	201.36	97.70	74.36
510	66.06	90.70	102.30	710	204.41	96.20	72.40
515	69.25	89.65	98.81	715	207.41	94.60	70.40
520	72.50	89.50	96.90	720	210.36	92.90	68.30
525	75.79	90.43	96.78	725	213.26	91.10	66.30
530	79.13	92.20	98.00	730	216.12	89.40	64.40
535	82.52	94.46	99.94	735	218.92	88.00	62.80
540	85.95	96.90	102.10	740	221.66	86.90	61.50
545	89.41	99.16	103.95	745	224.36	85.90	60.20
550	92.91	101.00	105.20	750	227.00	85.20	59.20
555	96.44	102.20	105.67	755	229.58	84.80	58.50
560	100.00	102.80	105.30	760	232.11	84.70	58.10
565	103.58	102.92	104.11	765	234.59	84.90	58.00
570	107.18	102.60	102.30	770	237.01	85.40	58.20
575	110.80	101.90	100.15	775	239.37	86.10	58.50
580	114.44	101.00	97.80	780	241.67	87.00	59.10

TABLE 9·4a COMPUTATION FORM

I.C.I. Coordinates Illuminant *A* (2854°K.)

Sample .... Source of Trans. Data ..... .
Submitted by .. . .

λ	$(\bar{x}E)_\lambda$	$(\bar{y}E)_\lambda$	$(\bar{z}E)_\lambda$	T_λ	$(\bar{x}ET)_\lambda$	$(\bar{y}ET)_\lambda$	$(\bar{z}ET)_\lambda$
380	1		6	0.			
90	5		23	.			
400	19	1	93	.			
10	71	2	340	.			
20	262	8	1256	.			
30	649	27	3167	.			
40	926	61	4647	.			
450	1031	117	5435	.			
60	1019	210	5851	.			
70	776	362	5116	.			
80	428	622	3636	.			
90	160	1039	2324	.			
500	27	1792	1509	.			
10	57	3080	969	.			
20	425	4771	525	.			
30	1214	6322	309	.			
40	2313	7600	162	.			
550	3732	8568	75	.			
60	5510	9222	36	.			
70	7571	9457	21	.			
80	9719	9228	18	.			
90	11579	8540	12	.			
600	12704	7547	10	.			
10	12669	6356	4	.			
20	11373	5071	3	.			
30	8980	3704		.			
40	6558	2562		.			
650	4336	1637		.			
60	2628	972		.			
70	1448	530		.			
80	804	292		.			
90	404	146		.			
700	209	75		.			
10	110	40		.			
20	57	19		.			
30	28	10		.			
40	14	6		.			
750	6	2		.			
60	4	2		.			
70	2			.			
Sums →	109828	100000	35547	Sums → $S = X+Y+Z$	$X =$	$Y =$	$Z =$
x_w, y_w, z_w	0.4476	0.4075	0.1449				
x, y, and z → ($x = X/S$, $y = Y/S$, $z = Z/S$)							

Planck 2854°K., C_2 = 14,380 Computed by . . ... Checked by . .... .
Planck 2848°K., C_2 = 14,350

TABLE 9·4b COMPUTATION FORM

I.C.I. Coordinates **Illuminant *C* (Davis-Gibson, 2854 to 6800°K.)**

Sample .. .. Source of Trans. Data... .
Submitted by.. ....

λ	$(\bar{x}E)_\lambda$	$(\bar{y}E)_\lambda$	$(\bar{z}E)_\lambda$	T_λ	$(\bar{x}ET)_\lambda$	$(\bar{y}ET)_\lambda$	$(\bar{z}ET)_\lambda$
380	4		20	0.			
90	19		89	.			
400	85	2	404	.			
10	329	9	1570	.			
20	1238	37	5949	.			
30	2997	122	14628	.			
40	3975	262	19938	.			
450	3915	443	20638	.			
60	3362	694	19299	.			
70	2272	1058	14972	.			
80	1112	1618	9461	.			
90	363	2358	5274	.			
500	52	3401	2864	.			
10	89	4833	1520	.			
20	576	6462	712	.			
30	1523	7934	388	.			
40	2785	9149	195	.			
550	4282	9832	86	.			
60	5880	9841	39	.			
70	7322	9147	20	.			
80	8417	7992	16	.			
90	8984	6627	10	.			
600	8949	5316	7	.			
10	8325	4176	2	.			
20	7070	3153	2	.			
30	5309	2190		.			
40	3693	1443		.			
650	2349	886		.			
60	1361	504		.			
70	708	259		.			
80	369	134		.			
90	171	62		.			
700	82	29		.			
10	39	14		.			
20	19	6		.			
30	8	3		.			
40	4	2		.			
750	2	1		.			
60	1	1		.			
70	1			.			
Sums ⟶	98041	100000	118103	Sums ⟶ $S = X + Y + Z$	$X =$	$Y =$	$Z =$
x_w, y_w, z_w	0.3101	0.3163	0.3736				
x, y, and z ⟶ ($x = X/S$, $y = Y/S$, $z = Z/S$)							

Planck 2854°K., $C_2 = 14{,}380$
Planck 2848°K., $C_2 = 14{,}350$

Computed by........... Checked by . . . ..

representative of average daylight such as that from a completely overcast sky. Illuminant A is an incandescent lamp operated at a color temperature of 2854°K. on the international temperature scale ($C_2 = 14{,}380$). Illuminant B is obtained by using this same lamp in combination with a two-cell Davis-Gibson liquid filter designed to give a color temperature of about 5000°K. Illuminant C is obtained similarly and results in a source of nearest color temperature about 6800°K. These illuminants are recommended for general use, or whenever there is no special reason for using some other source. Table 9·3 gives the relative spectral irradiance of illuminants A, B, and C. Tables 9·4a and 9·4b give computation forms for evaluation of the colors of non-self-luminous specimens that transmit, scatter, or reflect incident light. Table 9·4a refers to illuminant A; Table 9·4b, to illuminant C. Illuminant B is relatively little used except in Great Britain.

9·8 Computation of luminous reflectance and chromaticity coordinates. The fundamental nature of the tristimulus specification of color permits it to be used as a common denominator by means of which colorimeters involving color standards of glass, plastic, or solutions, or systems of material color standards, transparent and opaque, may be intercompared. In order to demonstrate how the I.C.I. standard observer and coordinate system may be used for this purpose, four printing inks, red purple, greenish yellow, greenish blue, and blue, have been evaluated, and the steps are reproduced here in detail. Figure 9·4 shows spectral reflectances of these four printing inks obtained on a recording spectrophotometer. A square on each curve sheet has been printed with the corresponding ink, and except for small variations in film thickness, difficult to control, should correspond closely, for a few months, at least, to the specimens actually measured. Table 9·5a gives the spectral reflectances read from the originals of the curves of Fig. 9·4. These reflectances apply to the specimens measured except for small wavelength-scale and photometric-scale corrections which have not been applied. Table 9·5b gives for the greenish yellow specimen the products indicated on the form for computation of luminous reflectance, Y, and chromaticity coordinates, x, y, z, under standard illuminant C; see Table 9·4b. The sums of these products are the tristimulus values, X, Y, Z. The luminous reflectance is found as $Y/100{,}000$; and the chromaticity coordinates, x, y, z, are found by dividing X, Y, and Z, respectively, by the corresponding sum, $X + Y + Z$. Table 9·5c lists these results for all four printing-ink specimens. Figure 9·5 is the (x, y)-chromaticity diagram on which have been plotted large dots to represent these chromaticity coordinates, x, y. The inks used to print these dots also correspond to the specimens measured.

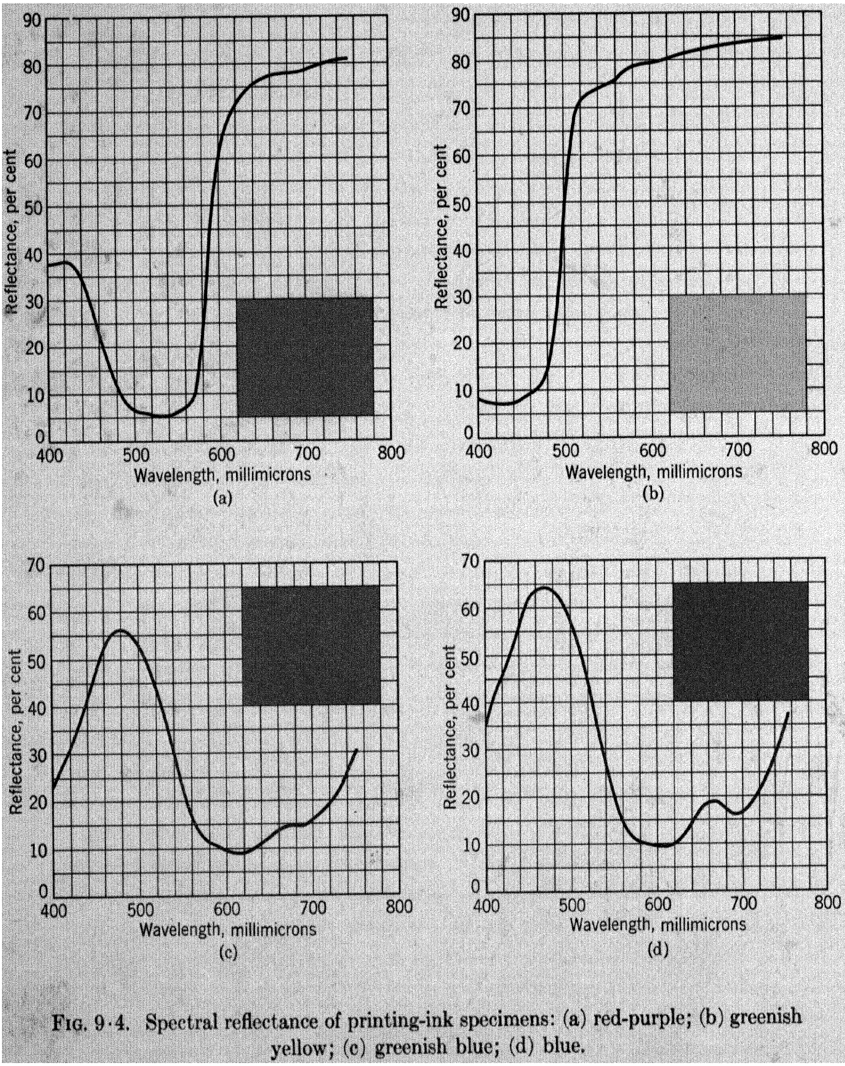


FIG. 9·4. Spectral reflectance of printing-ink specimens: (a) red-purple; (b) greenish yellow; (c) greenish blue; (d) blue.

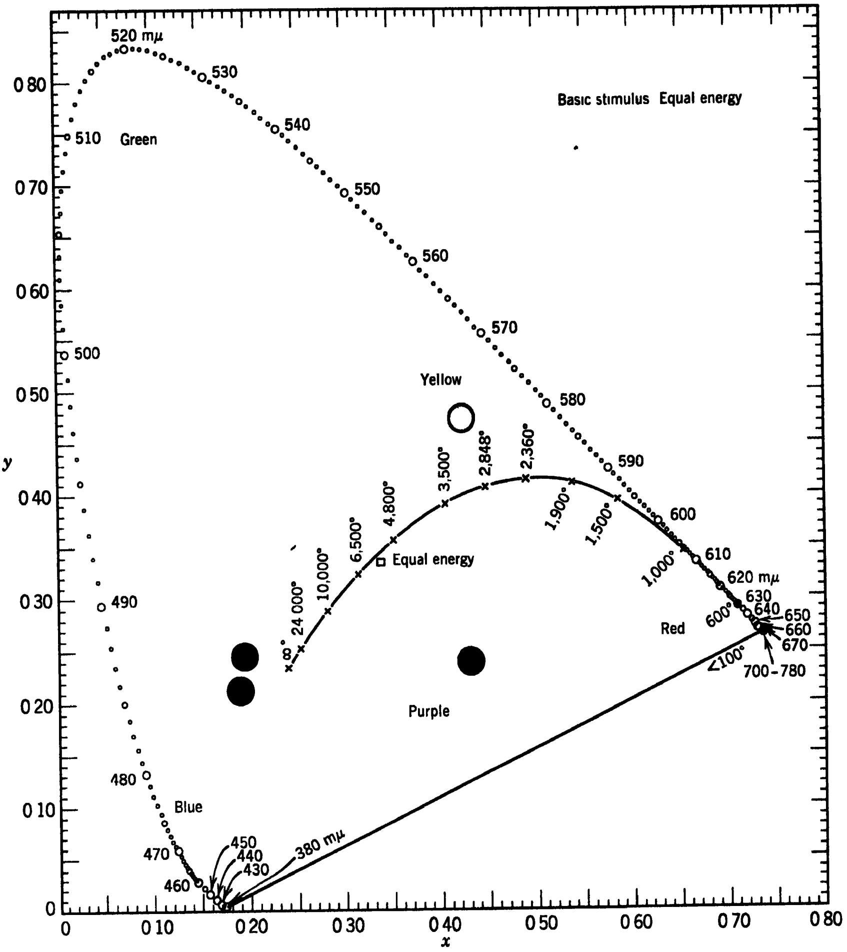


FIG. 9·5. Points representing the colors of four printing-ink specimens whose spectral reflectances are shown in Fig. 9·4. The colors of the ideal closed-cavity radiator are also shown, the temperatures of the radiators being indicated in degrees Kelvin. The smooth curve connecting these points is often called the Planckian locus.

TABLE 9·5a SPECTRAL REFLECTANCES OF FOUR PRINTING-INK SPECIMENS

Wavelength, mμ	*Spectral Reflectance Relative to Magnesium Oxide*			
	Red Purple	Greenish Yellow	Greenish Blue	Blue
380	0.375 *	0.091 *	0.150 *	0.230 *
390	.375 *	.089 *	.187 *	.293 *
400	.376	.085	.228	.354
410	.379	.079	.269	.415
420	.381	.077	.306	.458
430	.373	.076	.353	.505
440	.345	.077	.407	.563
450	.295	.086	.467	.616
460	.235	.095	.520	.639
470	.174	.108	.552	.645
480	.120	.145	.560	.635
490	.083	.250	.548	.608
500	.066	.445	.523	.568
510	.061	.635	.483	.508
520	.057	.708	.432	.438
530	.054	.725	.363	.353
540	.055	.733	.292	.272
550	.062	.743	.220	.198
560	.071	.752	.162	.145
570	.095	.768	.128	.117
580	.220	.782	.113	.106
590	.440	.787	.102	.102
600	.597	.790	.093	.098
610	.676	.793	.088	.097
620	.715	.798	.088	.103
630	.739	.803	.098	.122
640	.756	.809	.110	.147
650	.768	.814	.124	.172
660	.776	.818	.136	.187
670	.780	.822	.145	.186
680	.782	.824	.147	.172
690	.783	.827	.149	.162
700	.788	.829	.160	.169
710	.794	.832	.177	.192
720	.799	.833	.196	.221
730	.805	.835	.218	.256
740	.809	.836	.258	.304
750	.812	.837	.298	.362
760	.815 *	.838 *	.338 *	.422 *
770	.817 *	.839 *	.375 *	.484 *

* Extrapolated.

Table 9·5b Computation of Tristimulus Values, X, Y, Z, and Chromaticity Coordinates, x, y, for the Greenish-yellow Printing-ink Specimen under Illuminant C

(The computation form given as Table 9·4b has been used.)

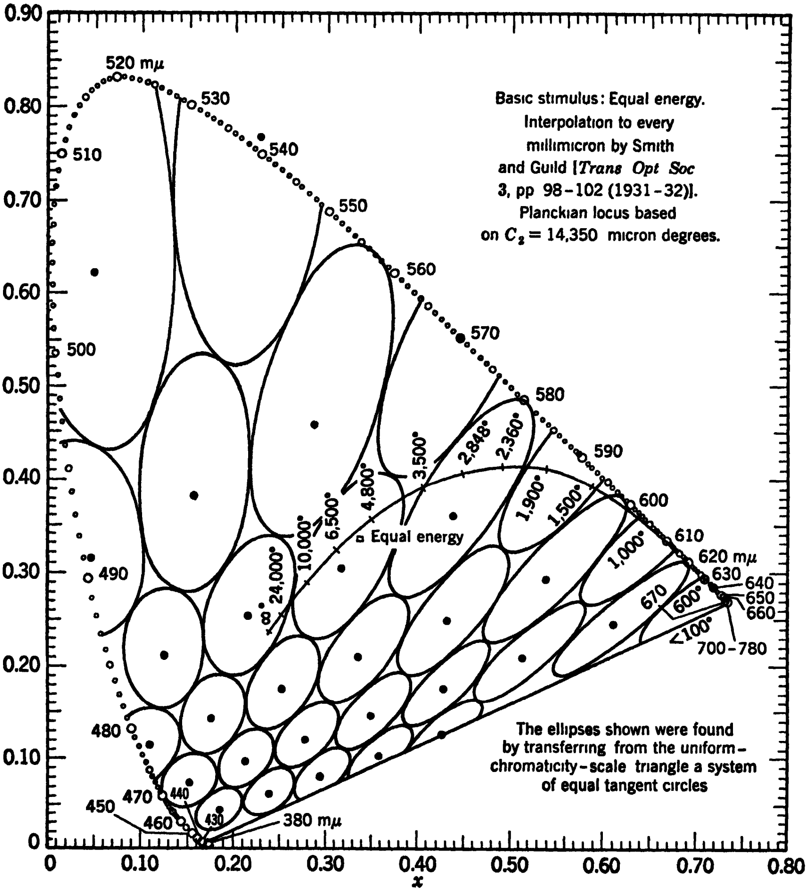
Wavelength, $m\mu$	*Reflectance* (R)	*Tristimulus Values*		
		$RE_C\bar{x}$	$RE_C\bar{y}$	$RE_C\bar{z}$
380	0.091	0		2
390	.089	2		8
400	.085	7	0	34
410	.079	26	1	124
420	.077	95	3	458
430	.076	228	9	1112
440	.077	306	20	1535
450	.086	337	38	1775
460	.095	319	66	1833
470	.108	245	114	1617
480	.145	161	235	1372
490	.250	91	590	1319
500	.445	23	1513	1274
510	.635	57	3069	965
520	.708	408	4575	504
530	.725	1104	5752	281
540	.733	2041	6706	143
550	.743	3182	7305	64
560	.752	4422	7400	29
570	.768	5623	7025	15
580	.782	6582	6250	13
590	.787	7070	5215	8
600	.790	7070	4200	6
610	.793	6602	3312	2
620	.798	5642	2516	2
630	.803	4263	1759	0
640	.809	2988	1167	
650	.814	1912	721	
660	.818	1113	412	
670	.822	582	213	
680	.824	304	110	
690	.827	141	51	
700	.829	68	24	
710	.832	32	12	
720	.833	16	5	
730	.835	7	3	
740	.836	3	2	
750	.837	2	1	
760	.838	1	1	
770	.839	1	0	
Totals	X, Y, Z	63,076	70,395	14,495
Fractions	x, y, z	0.4263	0.4758	0.0980

TABLE 9·5c TRISTIMULUS VALUES, X, Y, Z, UNDER ILLUMINANT C, LUMINOUS REFLECTANCE RELATIVE TO MAGNESIUM OXIDE, Y/Y_0, AND CHROMATICITY COORDINATES, x, y, COMPUTED FROM THE SPECTRAL REFLECTANCES OF FOUR PRINTING-INK SPECIMENS AS IN TABLE 9·5b

Hue Designation of Specimen	*Tristimulus Values, Arbitrary Units*			*Luminous Reflectance,*	*Chromaticity Coordinates*	
	X	Y	Z	Y/Y_0	x	y
Red purple	39788	22124	30570	0.221	0.430	0.239
Greenish yellow	63076	70395	14495	.704	.426	.476
Greenish blue	19003	24245	54529	.242	.194	.248
Blue	21948	24633	69010	.246	.190	.213

Comparison of Fig. 9·5 with Fig. 9·3 shows that the chromaticity points of the four printing-ink specimens correspond to the hue designations red purple, greenish yellow, and blue. This accords well with the color designations found by visual inspection of the specimens. Furthermore, it will be noted that one of the blues is greener than the other. The position of the chromaticity point is in accord with the greener hue. Note also that the greenish yellow is a much lighter color than the red purple or either of the blues; this accords with the luminous reflectances (compare 0.704 with 0.221, 0.242, and 0.246 in Table 9·5c).

9·9 Chromaticity spacing. In the interpretation of the importance of chromaticity differences based upon separation of the points representing the two chromaticities in the (x, y) diagram a warning is necessary. This diagram is considerably expanded in the green portion relative to the other portions, much as the Mercator projection of the earth's surface is expanded near the poles. Thus, two points separated by a given distance in the green portion of this diagram correspond to chromaticities that are much harder to distinguish under ordinary viewing conditions than two chromaticities separated by the same amount in other portions of the diagram. Furthermore the bluish purple portion of the diagram is correspondingly compressed. The system of ellipses shown on Fig. 9·6 serves to indicate approximately the metric properties of the (x, y) diagram. Under moderately good observing conditions, the distances from the central point of each ellipse to any point on its boundary correspond approximately to one hundred times the chromaticity difference just perceptible with certainty. These ellipses were drawn from a review of the literature in 1936 (*46*, *47*), and subsequent extensive work published by Wright in 1941 (*114*, *115*) and by MacAdam in 1942 (*62*, *63*) has corroborated the essential correctness of the indicated chromaticity spacing. Figure 9·6 not only indicates the extent to which the green portion of the diagram is expanded, and the bluish purple compressed, but also indicates that, in general, the chromatic importance of a distance on the (x, y) diagram is a function both of the position of the central point and the direction of the deviation from it.



Basic stimulus: Equal energy. Interpolation to every millimicron by Smith and Guild [*Trans Opt Soc* 3, pp 98-102 (1931-32)]. Planckian locus based on C_2 = 14,350 micron degrees.

The ellipses shown were found by transferring from the uniform-chromaticity-scale triangle a system of equal tangent circles

FIG. 9·6. Chromaticity spacing in the (x, y) diagram. Between any center and any point on the corresponding ellipse there are approximately 100 just noticeable chromaticity steps (*47*).

9·10 Short-cuts in computing tristimulus values. The labor of computing X, Y, Z, or Y, x, y, corresponding to pairs of spectrophotometric curves to see how the colors of the corresponding specimens compare is considerable. Often the degree of metamerism exhibited by the pair is sufficiently small that the comparison can be made directly from the curves themselves, and much product-control work can be handled in this way. There is still frequent need, in the establishment of color standards and tests for conformity to those standards, to compute the tristimulus values, X, Y, Z, and two short-cut methods of considerable value in reducing data obtained by the recording spectrophotometer have been developed.

9·11 Selected ordinate method. In this method the spectrophotometric curve, instead of being read at every 5 or 10 mμ, is read at a series of selected ordinates different for each illuminant. Instead of multiplying by the tristimulus values of the spectrum of the illuminant, $E\bar{x}_\lambda$, $E\bar{y}_\lambda$, $E\bar{z}_\lambda$, the selected ordinates are spaced proportionately closer in the wavelength regions where the tristimulus values are higher, and the corresponding readings of spectral reflectance are simply added. Table 9·6 gives (*16*, *36*) selected ordinates for illuminant A (incandescent lamp light) and illuminant C (average daylight). Table 9·7 gives the spectral reflectances of the greenish yellow printing-ink specimen read from Fig. 9·4b for the selected ordinates for illuminant C together with the sums of these spectral reflectances, both for ten ordinates and for thirty. It will be noted that, after applying the multiplying factors listed in Table 9·6, the tristimulus values, X, Y, Z, for the greenish yellow printing-ink specimen are found again to a close approximation (compare 0.630, 0.704, and 0.145 from Table 9·7 with 0.631, 0.704, and 0.145, respectively, from Table 9·5c).

Ten selected ordinates sometimes give significant information (see Table 9·7); thirty selected ordinates often are sufficient (as above); and one hundred selected ordinates are sufficient for all but a few very irregular spectral distributions (such as produced by gaseous discharge tubes). These wavelengths are available for many illuminants in other publications (*10*, *16*, *36*). Nickerson (*78*) has published a study of the reliability of the selected-ordinate method of computation.

9·12 Semigraphical centroid method. In this method, due to Robinson (*98*), the spectrophotometric curve is divided into a number of segments, each segment covering a wavelength interval for which the centroid has already been computed, and a straight-line approximation is drawn in for each segment. A few curves are sufficiently simple that straight-line approximations to 1 or 2 segments give significant information; 4 to 8 segments are most often used; segments as small as 20 mμ are occasionally required. For curves so irregular as to require segments smaller than 20 mμ this method is scarcely shorter than the original method of summation over wavelength intervals of 5 or 10 mμ. The method is based upon two principles. First, the spectral distribution of each primary may be considered a variable mass distribution along the spectrophotometric curve. If the curve is a straight line for a certain

TABLE 9·6 SELECTED ORDINATES (IN MILLIMICRONS) FOR COMPUTING TRISTIMULUS VALUES, X, Y Z, FOR SPECIMENS UNDER STANDARD ILLUMINANTS A AND C (*16*)

	For Illuminant A			*For Illuminant C*		
Number	*X*	*Y*	*Z*	*X*	*Y*	*Z*
1	444.0	487.8	416.4	424.4	465.9	414.1
2 *	516.9 *	507.7 *	424.9 *	435.5 *	489.4 *	422.2 *
3	544.0	517.3	429.4	443.9	500.4	426.3
4	554.2	524.1	432.9	452.1	508.7	429.4
5 *	561.4 *	529.8 *	436.0 *	461.2 *	515.1 *	432.0 *
6	567.1	534.8	438.7	474.0	520.6	434.3
7	572.0	539.4	441.3	531.2	525.4	436.5
8 *	576.3 *	543.7 *	443.7 *	544.3 *	529.8 *	438.6 *
9	580.2	547.8	446.0	552.4	533.9	440.6
10	583.9	551.7	448.3	558.7	537.7	442.5
11 *	587.2 *	555.4 *	450.5 *	564.1 *	541.4 *	444.4 *
12	590.5	559.1	452.6	568.9	544.9	446.3
13	593.5	562.7	454.7	573.2	548.4	448.2
14 *	596.5 *	566.3 *	456.8 *	577.3 *	551.8 *	450.1 *
15	599.4	569.8	458.8	581.3	555.1	452.1
16	602.3	573.3	460.8	585.0	558.5	454.0
17 *	605.2 *	576.9 *	462.9 *	588.7 *	561.9 *	455.9 *
18	608.0	580.5	464.9	592.4	565.3	457.9
19	610.9	584.1	467.0	596.0	568.9	459.9
20 *	613.8 *	587.9 *	469.2 *	599.6 *	572.5 *	462.0 *
21	616.9	591.8	471.6	603.3	576.4	464.1
22	620.0	595.9	474.1	607.0	580.5	466.3
23 *	623.3 *	600.1 *	476.8 *	610.9 *	584.8 *	468.7 *
24	626.9	604.7	479.9	615.0	589.6	471.4
25	630.8	609.7	483.4	619.4	594.8	474.3
26 *	635.3 *	615.2 *	487.5 *	624.2 *	600.8 *	477.7 *
27	640.5	621.5	492.7	629.8	607.7	481.8
28	646.9	629.2	499.3	636.6	616.1	487.2
29 *	655.9 *	639.7 *	508.4 *	645.9 *	627.3 *	495.2 *
30	673.5	659.0	526.7	663.0	647.4	511.2
Multiplying factors						
30 ordinates	0.03661	0.03333	0.01185	0.03268	0.03333	0.03938
10 ordinates	.10984	.10000	.03555	.09804	.10000	.11812

* Values for calculation with 10 selected ordinates.

TABLE 9·7 SPECTRAL REFLECTANCES OF GREENISH YELLOW PRINTING-INK SPECIMEN (SEE FIG. 9·4b) READ FOR THE SELECTED ORDINATES FOR ILLUMINANT *C* (SEE TABLE 9·6)

Number	*X*		*Y*		*Z*	
1	0.076		0.102		0.078	
2 *	.076	0.076	.240	0.240	.076	0.076
3	.079		.460		.076	
4	.088		.615		.075	
5 *	.096	.096	.683	.683	.075	.075
6	.117		.711		.076	
7	.727		.720		.076	
8 *	.739	.739	.725	.725	.077	.077
9	.745		.728		.077	
10	.750		.731		.078	
11 *	.758	.758	.735	.735	.080	.080
12	.765		.738		.082	
13	.772		.742		.084	
14 *	.779	.779	.745	.745	.086	.086
15	.783		.747		.088	
16	.785		.750		.089	
17 *	.787	.787	.755	.755	.091	.091
18	.788		.760		.093	
19	.789		.765		.095	
20 *	.790	.790	.772	.772	.097	.097
21	.791		.778		.100	
22	.792		.782		.103	
23 *	.793	.793	.785	.785	.106	.106
24	.795		.787		.110	
25	.797		.788		.119	
26 *	.800	.800	.790	.790	.133	.133
27	.803		.792		.155	
28	.807		.796		.210	
29 *	.812	.812	.802	.802	.342	.342
30	.819		.812		.650	
Totals	19.298	6.430	21.136	7.032	3.677	1.163
Times factor	0.630	0.630	0.704	0.703	0.145	0.137

Values for calculation with 10 selected ordinates.

Table 9·8a Wavelength Centroids in the Spectrum of Illuminant *A* from 400 to 700 mμ taken in 1, 2, 4, 8, and 15 Intervals with Respect to *X*, *Y*, and *Z*

Wave-length, mμ	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_3	Y_4	Y_5	Z_1	Z_2	Z_3	Z_4	Z_5
400															
	414.3					414.4					414.3				
420		429.8					431.8					430.0			
	432.2					433.5					432.3				
440			450.0					465.3					452.1		
	450.6					452.5					450.9				
460		458.4					467.4					459.8			
	469.1					472.3					469.7				
480				514.8					532.5					461.8	
	486.8					492.2					489.0				
500		499.0					507.0					494.7			
	515.9					512.1					508.8				
520			544.5					534.4					498.7		
	533.0					531.2					528.6				
540		547.1					542.5					532.6			
	551.9					550.8					548.0				
560					595.2					572.1					462.1
	571.4					570.5					569.2				
580		583.1					579.8					576.7			
	590.0					590.2					589.6				
600			601.6					593.2					582.3		
	610.3					609.8					608.4				
620		618.3					617.1					612.3			
	629.6					629.4					627.7				
640				608.9					598.1					582.5	
	649.0					648.9					644.2	644.2	644.2		
660		654.0					653.8								
	668.6					668.5									
680			656.4					656.1							
	688.3	688.3				688.3	688.3								
700															
Multiplying factor		1.0983						1.0000					0.3555		

TABLE 9·8b WAVELENGTH CENTROIDS IN THE SPECTRUM OF ILLUMINANT C FROM 400 TO 700 Mμ TAKEN IN 1, 2, 4, 8, AND 15 INTERVALS WITH RESPECT TO X, Y, AND Z

Wave-length, mμ	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_3	Y_4	Y_5	Z_1	Z_2	Z_3	Z_4	Z_5
400	414.4	429.6	447.2	486.2	565.3	414.5	431.7	463.1	526.6	557.0	414.4	429.8	449.1	455.0	455.1
420	432.1					433.3					432.2				
440	450.2	457.0				452.0	466.0				450.4	458.3			
460	468.7					471.9					469.3				
480	486.5	495.4	542.0			491.7	505.0	531.0			488.5	492.9	495.5		
500	515.6					511.6					508.2				
520	532.8	546.6				531.1	541.8				528.5	532.2			
540	551.7					550.6					547.9				
560	571.1	581.8	598.0	603.7		568.3	577.4	588.6	592.2		568.9	575.4	579.7	579.8	
580	590.6					589.8					589.2				
600	610.1	617.7				609.6	616.4				608.2	611.8			
620	629.4					629.2					627.7				
640	648.9	653.5	655.5			648.8	653.3	655.2			643.8	643.8	643.8		
660	668.4					668.4									
680	688.0	688.0				688.0	688.0								
700															
Multiplying factor			0.9804					1.0000					1.1810		

wavelength interval, the center of gravity for that interval must fall somewhere on the line, and the wavelength of this center of gravity can be computed from the spectral distribution of the primary itself. Tables 9·8a and b give the wavelength centroids for X, Y, and Z in the spectra of illuminants A and C from 400 to 700 mμ taken in 1, 2, 4, 8, and 15 intervals.

The second principle is that the center of gravity of a reflectance curve over any interval lies somewhere on the straight line connecting the centers of gravity of any two subdivisions of that interval. The method is therefore simply to locate the point on the straight-line approximations for each segment that corresponds to the wavelength in Table 9·8; then connect these points in pairs by straight lines, locate new points at the wavelengths given in Table 9·8 for the double intervals, and so on until one straight line for the entire spectrum is found. The tristimulus value is then found by reading on the scale of spectral reflectance the ordinate on this line corresponding to the wavelength given in the final columns of Table 9·8 for X, Y, or Z, as the case may be, and applying the indicated multiplying factor.

Figure 9·7 shows the application of this method to the spectrophotometric curve for the greenish yellow printing-ink specimen to obtain its luminous reflectance, Y/Y_0, relative to magnesium oxide for illuminant C. Five segments have been used, 400 to 440, 440 to 480, 480 to 520, 520 to 560, and 560 to 700 mμ. The straight-line approximations to these segments are shown by light solid lines. The points on these straight lines for the wavelength centroids given in Table 9·8b under Y_2 (431.7, 466.0, 505.0, 541.8) and under Y_4 (592.2) are shown by circles. The centers of these circles for the first four intervals are connected in pairs by dashed lines, 431.7 with 466.0, and 505.0 with 541.8. The points on these dashed lines corresponding to the wavelength centroids given under Y_3 (463.1 and 531.0) are also shown by circles and have also been connected by a dashed line. The point on this dashed line corresponding to the centroid (526.6) given under Y_4 of Table 9·8b is shown by a circle and is connected by a dashed line with the circle plotted to correspond to the other centroid (592.2) given under Y_4. The point on this dashed line corresponding to the centroid, Y_5, of the table (557.0) is indicated by a square. The reflectance corresponding to this square multiplied by the multiplying factor (1.0000) given in Table 9·8b is the desired evaluation of luminous reflectance, Y/Y_0, relative to magnesium oxide, of the greenish yellow printing-ink specimen for illuminant C. This is seen to be 0.70_2, which is in good agreement with that (0.704) found by 10-mμ summation (Table 9·5b).

Similar evaluation of X and Z by the centroid method gives 0.63_2 and 0.14_4, which differ by only one in the third decimal from the values (0.631

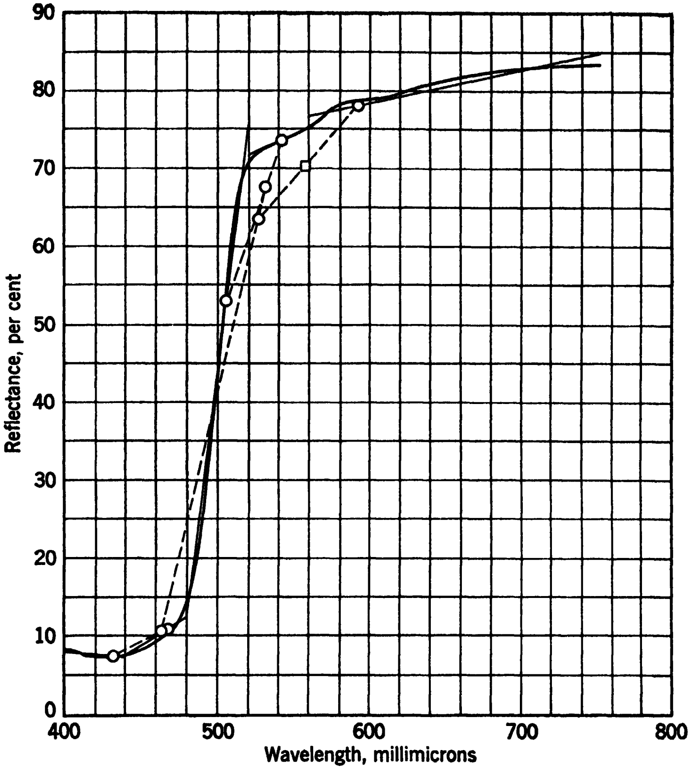


Fig. 9·7. Evaluation of the luminous reflectance of the greenish yellow printing-ink specimen from the curve of Fig. 9·4b by the Robinson centroid method (*98*). Wavelength centroids were taken from Table 9·8b for illuminant *C*.

and 0.145) obtained in Table 9·5b. This suggests that the straight lines drawn as approximations to the five segments of the curve for the greenish yellow printing-ink specimen are by good fortune unusually close approximations because it is more usual to find errors of 3 or 4 in the third decimal by this method. For many purposes, however, errors of this size are quite tolerable, and the superior speed and simplicity of the method recommend it in these cases. Note that the curve itself does not have to be read at all.*

9·13 Tristimulus colorimeters. Tristimulus values, X, Y, Z, may be obtained by direct comparison of the unknown light with an optical mixture of three primary lights in a divided photometric field. Since the primaries of the I.C.I. standard colorimetric coordinate system are imaginary, such a tristimulus colorimeter cannot be made to read directly. It must be calibrated by measurements of four known stimuli, and then may yield tristimulus values, X, Y, Z, by a transformation the reverse of that indicated in equation (9·3). Since, except by accident, the color matches set up in a tristimulus colorimeter designed to cover any substantial part of the color totality with a single set of primaries exhibit serious metamerism, the field has to be relatively small, such as to subtend about 2 degrees at the observer's eye; and this restriction to a small angular size of field severely limits the precision of setting compared to what is possible by direct comparison of large specimens in daylight. Furthermore, the metamerism also prevents one normal observer from getting the same reading as another except by accident. If a reasonable approximation to the standard values of X, Y, and Z is to be assured, either the readings of a group of five or ten observers must be averaged, or a color standard yielding a spectral composition similar to that of the unknown specimen must be used. It will be seen that tristimulus colorimeters give but poor information regarding the unknown specimen. Their application to product-control problems is negligible, but because of the ease of calibration and simplicity of the theory they are very useful visual research tools. Tristimulus colorimeters have been built and described by Guild (*30*), Wright (*111*), Verbeek (*108*), Donaldson (*21*), and Newhall (*74*). The Wright instrument has spectrum primaries; the other four have primaries formed by combining a light source with glass filters. To the Guild and Wright instruments we owe our accurate information regarding the properties of the normal visual system which have been expressed in terms of the standard observer.

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* An integrating attachment to the General Electric recording spectrophotometer has recently become commercially available; see H. R. Davidson and L. W. Imm, *J. Optical Soc. Am.*, **39**, 633 (1949), and I. H. Godlove, *ibid.*, **39**, 633 (1949). This integrator computes the tristimulus values, X, Y, Z, of the specimen while its spectrophotometric curve is being recorded.

The foregoing instruments make up the comparison-field mixture by optical combination of light beams from different sources so that a sum of the separate effects is obtained. A similar optical effect is obtained if the beams are caused to fall upon the same portion of the retina in such rapid succession that a nonflickering spot of color is seen. The effect is that of a time-weighted average of the separate beams. A very simple and widely used tristimulus colorimeter is obtained by taking four discs that have been cut along a radius, interlocking them so as to expose a sector of each, and causing them to rotate on the spindle of a motor so rapidly that neither the separate sectors nor even flicker is perceived. Such an arrangement for combining colors by rotary mixture is called a Maxwell disc. The four discs provide the necessary three degrees of freedom in the adjustment for a match, and if the tristimulus values of the component discs be known (X_1, Y_1, Z_1; X_2, Y_2, Z_2; X_3, Y_3, Z_3; X_4, Y_4, Z_4), the tristimulus values of the mixture can be computed from the fractions of the total area occupied by the respective sectors, f_1, f_2, f_3, f_4:

$$\begin{aligned} X &= f_1X_1 + f_2X_2 + f_3X_3 + f_4X_4 \\ Y &= f_1Y_1 + f_2Y_2 + f_3Y_3 + f_4Y_4 \\ Z &= f_1Z_1 + f_2Z_2 + f_3Z_3 + f_4Z_4 \end{aligned} \tag{9·4}$$

Figure 9·8 shows a photograph of a Maxwell disc set up to duplicate the color of a cotton sample (*80*). If the discs are chosen anew for each kind of unknown color to be measured so as to be all fairly similar in color to the unknown, the spectral composition of the mixture color is usually sufficiently nonmetameric that no restriction to the central 2 degrees of the retina is required. Furthermore, any two normal observers with some experience at making the adjustment can check each other closely. The chief drawback of this simple arrangement for product-control work in color is the time lost in adjustment of the sector disc areas. The motor must be turned off, brought to a stop, the discs loosened and readjusted, the motor turned on and allowed to resume speed several times to obtain a final setting of reasonably good precision.

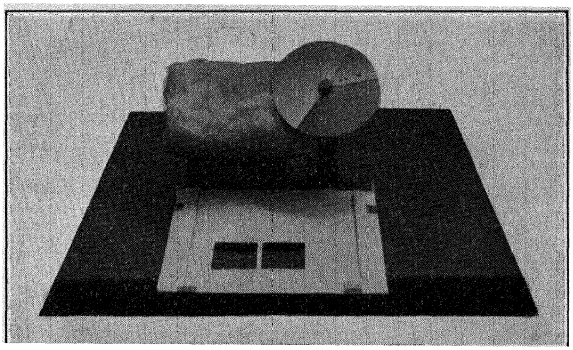


Fig. 9·8. Maxwell disc set up to measure the tristimulus values of a cotton sample. (Courtesy of Miss Dorothy Nickerson (*80*).)

Nickerson has described a disc colorimeter (*77*) that avoids the difficulties of the elementary Maxwell disc. Light reflected from the unknown specimen fills one-half of a photometric field, and that from a stationary sector disc fills the other. By having the observer look at the sector disc through a rapidly rotating glass wedge, each sector is presented to view in sufficiently quick succession that no flicker is produced; and at the same time the sectors, since they are stationary, may be continuously adjusted until a color match is obtained. Figure 9·9 shows a recent model of this disc colorimeter. A further advantage is obtained by extending the rotary scanning to the unknown specimen. In this way the average color of a notably nonuniform specimen such as that made up of coarse salt crystals may be obtained. The disc colorimeter is extensively used by the United States Department of Agriculture for the color-grading of food products and is well adapted to product-control colorimetry of many kinds.

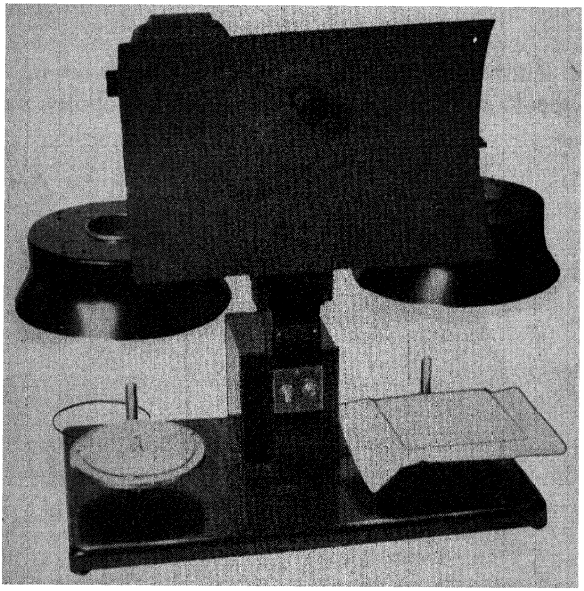


FIG. 9·9. Compact model disc colorimeter (Bausch and Lomb). Rotary scanning by a spinning optical wedge permits continuous adjustment of the disc proportions until a color match is obtained. (Courtesy of Bausch and Lomb Optical Company.)

9·14 Dominant wavelength and purity. Another form of identifying a combination of lights to specify a color, alternate to the tristimulus form, is to determine the luminance (photometric brightness) of one spot of light of fixed spectral composition (such as average daylight) and the luminance of a spot of light of continuously variable spectral composition separately identified (as by wavelength in the spectrum). In this way the requisite three degrees of freedom in adjustment to a color match are supplied. This form of identification leads naturally into a specification in which the luminance (*15*) of the unknown is given and the chromaticity is specified by two variables in polar coordinates.

One of these variables is an angle, the other a radius, and both can be computed from the chromaticity coordinates of the fixed spot of light, the variable spot of light, and the unknown, these coordinates serving to locate the respective positions in the Maxwell triangle. If the fixed light is nearly achromatic, the angle often correlates well with the hue of the color perception, and the radius fairly well with its saturation. The most fundamental way to specify the direction on the Maxwell triangle from the point representing the fixed light to the point representing the unknown light is by wavelength of the part of the spectrum required to make the match. If the unknown color can be matched by adding some part of the spectrum to the fixed light, it is said to have a spectral color, and the required wavelength is called its *dominant wavelength.* But if a color match is produced for the fixed light by adding some part of the spectrum to the unknown color, the unknown is said to be nonspectral, and the required wavelength is called the *complementary wavelength.* Either dominant wavelength or complementary wavelength may be obtained for the standard observer by drawing on the standard Maxwell triangle a straight line through the point representing the fixed light and that representing the unknown color, and then by reading the wavelength corresponding to the point at which this line extended intersects the locus of spectrum colors. If the unknown color is plotted between the fixed light and the spectrum, the intersection gives the dominant wavelength; but if the fixed light is represented by a point intermediate to the unknown and the intersection of the straight line with the spectrum locus, the intersection indicates the complementary wavelength.

The degree of approach of the unknown color to the spectrum color is commonly indicated by the ratio of the amount of the spectrum color to the total amount of the two-part combination; this ratio is called purity, and if the amounts are specified in luminance units, the ratio is called *luminance* (formerly colorimetric) *purity.* By far the most common convention, however, is to express the amounts in units of the excitation sum $X + Y + Z$; the resulting ratio is called *excitation purity* and corresponds simply to distance ratios on the Maxwell triangle of the standard colorimetric coordinate system (*36*, *44*, *91*). Formulas have been derived by Hardy (*36*) and MacAdam (*61a*) to convert from luminance purity to excitation purity, and the reverse.

Figure 9·10 indicates how dominant wavelength and excitation purity of the four printing-ink specimens would be found from their chromaticity coordinates, x, y, relative to illuminant C taken as the fixed light. Table 9·9 gives the dominant and complementary (C) wavelengths found as in Fig. 9·10 by the intersections of the straight lines with the spectrum locus. Table 9·9 also gives the excitation purities found by dividing the distance from the fixed point (illuminant *C*) to the specimen point by the total distance from the fixed point to the boundary (spectrum locus plus straight line connecting its extremes). Large-

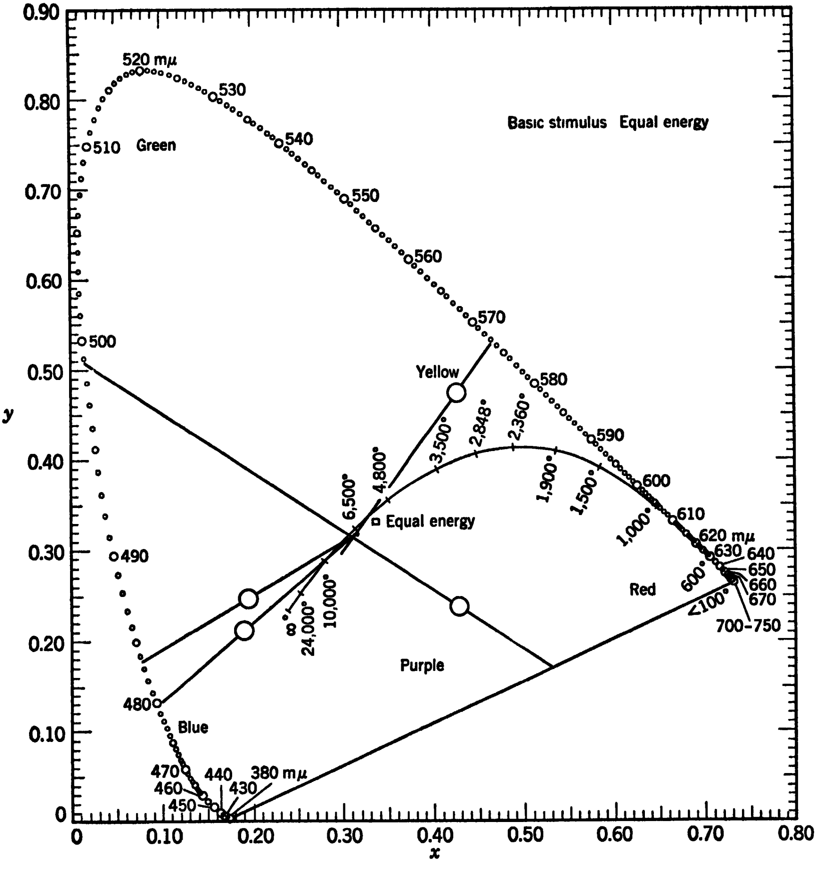


FIG. 9·10. Dominant wavelength and purity evaluated from the (x, y)-chromaticity diagram. The four points indicated by circles represent the colors of the four printing-ink specimens shown in Figs. 9·4 and 9·5.

scale charts for reading dominant wavelength and purity relative to illuminant *C* are provided in the Hardy *Handbook of Colorimetry* (*36*).

Apparatus for the direct measurement of dominant wavelength and luminance purity has been designed by Nutting (*83*) and by Priest (*90*). The degree of metamerism ordinarily obtained with such apparatus leaves it open to the same objections as have prevented tristimulus colorimeters with single sets of primaries from being useful for production control. There is a further disadvantage in the direct measurement of luminance purity in that the luminance of the spectrum component has to be determined relative to the luminance of the mixture by separate photometry. Since there is usually a large chromatic difference between these two fields, simple equality-of-brightness settings are not reliable, and an auxiliary flicker photometer, as in Priest's apparatus (*90*), must be used. This method has been found to exaggerate individual-observer differences; oftentimes observers will differ only slightly in the mixtures of spectrum light and fixed light that they find to be equivalent to an unknown color, but they will disagree importantly in their photometry of the components.

TABLE 9·9 DOMINANT WAVELENGTHS AND EXCITATION PURITIES OF FOUR PRINTING-INK SPECIMENS (SEE FIG. 9·4)

Hue Designation of Specimen	*Chromaticity Coordinates (from Table 9·5c)*		*Dominant Wavelength, mμ*	*Excitation Purity, Per Cent*
	x	*y*		
Red purple	0.430	0.239	498.9*C* *	53.7
Greenish yellow	.426	.476	573.2	74.0
Greenish blue	.194	.248	483.6	49.6
Blue	.190	.213	479.7	55.1

* *C* denotes complementary wavelength.

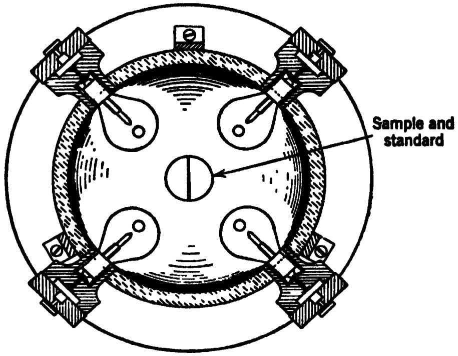
9·15 Guild vector method. By using two known fixed lights and one variable (spectrum) light, Guild (*31*) was able to determine chromaticity coordinates, x, y, without resort to auxiliary photometric devices. A match is first set up for the unknown by a mixture of one fixed light with some portion of the spectrum, the amounts of both components being adjustable by the observer. A straight line is drawn on the chromaticity diagram connecting the points representing the fixed light and the spectrum light. Then the process is repeated with a second fixed light, and a second straight line is drawn on the chromaticity diagram. The intersection of the two straight lines gives the chromaticity coordinates of the unknown. This method was used by Guild chiefly to supplement the tristimulus colorimeter by measuring unknowns whose chromaticities plot outside the triangle formed by plotting the points representing the primaries of the tristimulus colorimeter. Such unknowns may be evaluated on the tristimulus colorimeter only by the inconvenient expedient of mixing one of the primaries with the unknown. Since the matches obtained by the vector method are highly metameric, a group of several observers is required to obtain a representative result.

C·COLORIMETRY BY DIFFERENCE FROM A WORKING STANDARD

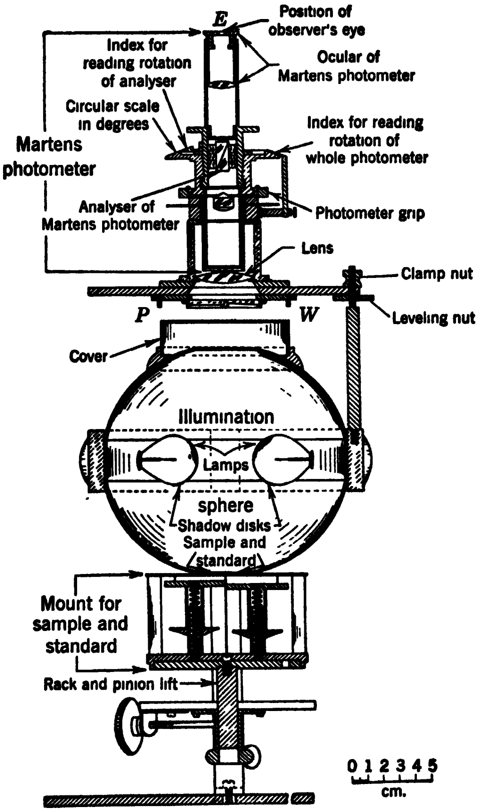
9·16 General principle. In the fundamental colorimetry of lights and objects a single standard is used for each class of specimen. Opaque surfaces are referred to a sufficiently thick layer of magnesium oxide deposited from the smoke of magnesium turnings or ribbon burning in air (*73*). Transparent objects, such as gelatin films and crystal or glass plates, are referred to the equivalent thickness of air; transparent solutions, to the equivalent thickness of distilled water or solvent. Self-luminous objects, such as fluorescent lamps, cathode-ray tubes, and incandescent lamps, are measured relative to one of the standard illuminants, usually illuminant *A* (*14*). The colors of specimens closely resembling the respective standards can be evaluated quite precisely and accurately; those differing radically in spectral composition, only with relative uncertainty. That is, near-white specimens, nearly clear glass plates, and incandescent lamps nearly equivalent to illuminant *A* present the simplest colorimetric problem; highly selective absorbers and emitters, like the rare-earth glasses and gaseous discharge tubes, present difficult measurement problems. In general, the greater the deviation in spectral composition between the unknown specimen and the standard, the greater the uncertainty of the result obtained by a visual or a photoelectric colorimeter.

The recording spectrophotometer has greatly extended the application of both visual and photoelectric colorimetry. It has supplied a practical way to calibrate working standards of color. If a fairly large group of specimens is at hand to be measured, say twenty or more, all of similar spectral composition, the most satisfactory way to measure them in the present state of colorimetric science is to evaluate one or two of them carefully by means of the spectrophotometer to serve as working standards, then obtain the color specifications of the remainder by visual or photoelectric determination of the difference between specimen and standard.

9·17 Martens photometer. One of the most useful visual devices for determining relative luminance is the Martens photometer. Figure 9·11 shows the Martens photometer combined with a diffuse illuminator to form the Priest-Lange reflectometer (*92*). It is intended for the measurement of luminous reflectance of opaque specimens relative to reflecting standards of similar spectral reflectance. It is also adaptable to the measurement of luminous transmittance of transparent plates relative to transmitting standards similar in spectral transmittance to the unknown. Finally, the Martens photometer, removed from the mounting may be used for the determination of the luminance of an un-



Horizontal equatorial section of illumination sphere


E
Position of observer's eye
Index for reading rotation of analyser
Ocular of Martens photometer
Circular scale in degrees
Index for reading rotation of whole photometer
Martens photometer
Analyser of Martens photometer
Photometer grip
Lens
Clamp nut
P
W
Leveling nut
Cover
Illumination
Lamps
sphere
Shadow disks
Sample and standard
Mount for sample and standard
Rack and pinion lift
0 1 2 3 4 5
cm.

Principal vertical section

FIG. 9·11. Vertical cross-section of the Priest-Lange (*92*) reflectometer, showing Martens photometer, diffuse illuminator, and specimen holder. Also (*above*) horizontal cross-section of the illuminator.

known self-luminous surface relative to a spectrally similar standard of known luminance. The superior usefulness of the Martens photometer arises from the convenience of the adjustment for equality of brightness between the two halves of the photometer field and from the fact that the dividing line between the half-fields is exceptionally narrow so that it is often invisible when a brightness match has been set.

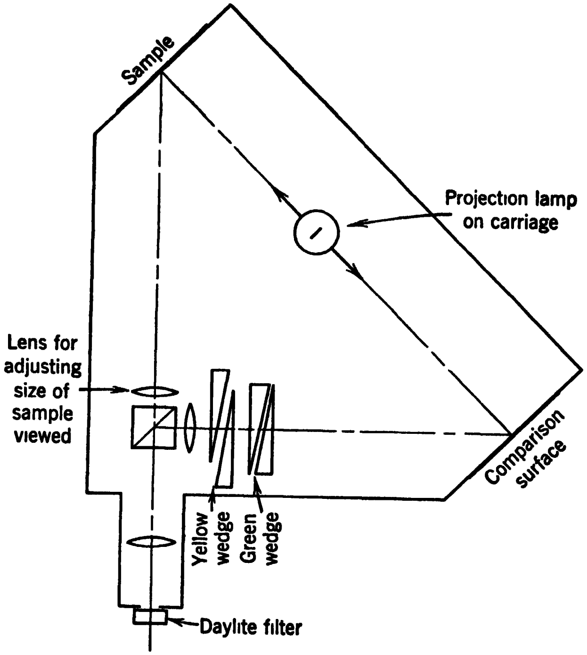

Sample
Projection lamp on carriage
Lens for adjusting size of sample viewed
Comparison surface
Yellow wedge
Green wedge
Daylite filter

FIG. 9·12. Schematic diagram of chromaticity-difference colorimeter (*48*).

9·18 Chromaticity-difference colorimeter. The determination of chromaticity coordinates, x, y, by comparison of the unknown specimen with a working standard of similar spectral reflectance can be carried out visually with high precision by means of a colorimeter described by Judd (*48*). The adjustment of the chromaticity of the comparison field to match the standard field is by two double wedges, one of greenish and the other of yellowish glass. Since the light from the comparison field must pass through both the yellow and the green wedge, some of the radiant energy being subtracted by each, it is sometimes called a subtractive colorimeter; see Fig. 9·12, which gives a schematic diagram. The standard and comparison fields are brought into juxtaposition by means of a Lummer-Brodhun cube having a double-trapezoid pattern subtending 9 × 13° at the observer's eye. The adjustment to near equality of brightness to facilitate detection of chromaticity differences is by movement of the projection lamp that illuminates both standard and comparison surfaces.

A substitution method is usually employed with this colorimeter, a match first being set up between the standard and comparison surfaces by adjustment of the wedges. Then the unknown specimen is substituted for the standard, and the wedges readjusted to restore the match.

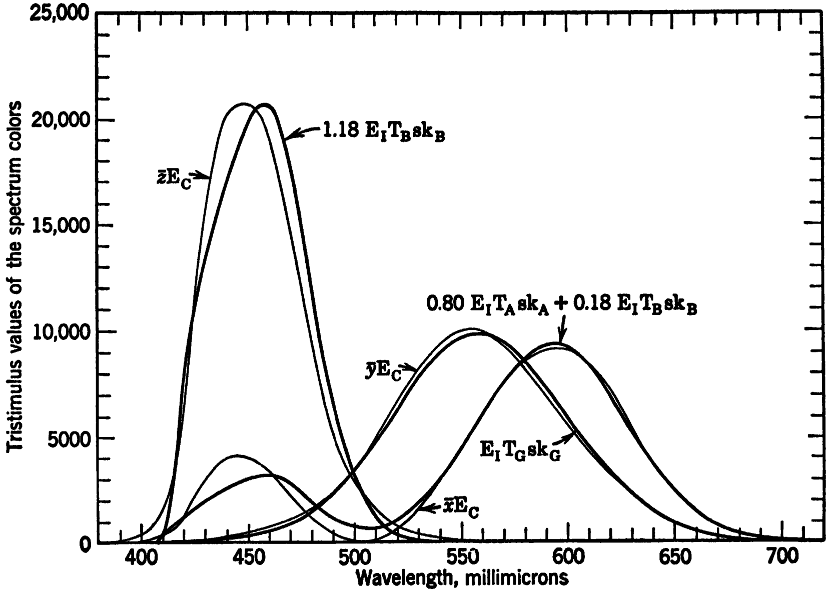


Fig. 9·13. Curves showing the degree to which the Hunter tristimulus filters (*41*) combined with incandescent lamp and barrier-layer cell duplicate the I.C.I. tristimulus values of the spectrum of illuminant *C*. (Taken from *Natl. Bur. Standards Circular* C429.)

The differences in wedge settings can be calibrated in terms of differences in the chromaticity coordinates, x, y, of the I.C.I. standard coordinate system, provided the spectral reflectances of the comparison surface are known approximately from the known spectral transmittances of the wedges. This calibration has been carried out for about 100 widely differing comparison surfaces. It has been found that the calibration is chiefly a function of the chromaticity coordinates, x, y, of the comparison surface; so calibrations for comparison surfaces intermediate in chromaticity to those already calibrated may usually be found satisfactorily by interpolation.

Because of the large patterned field of high luminance and the convenience of the brightness adjustment this instrument takes full advantage of the ability of the observer to detect small chromaticity differences. If specimen and comparison surfaces are similar in spectral composition, the settings for match may be repeated generally within

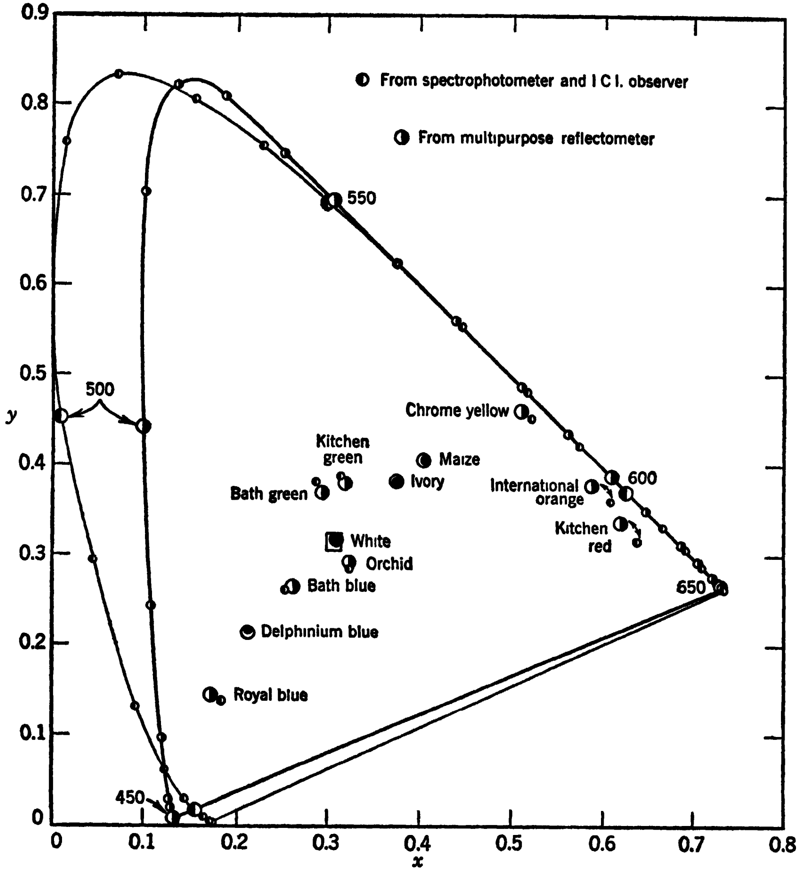


Fig. 9·14. Chromaticity discrepancies expected from the use of the multipurpose reflectometer with Hunter tristimulus filters (*41*). Measurements are all referred to magnesium oxide as the standard white. (Taken from *Natl. Bur. Standards Circular* C429.)

0.001 in chromaticity coordinates, x, y. The chromaticity-difference colorimeter has the disadvantage, however, of requiring a skilled operator. Furthermore the calibrations, carried out in accord with Table 9·4b, are time-consuming; and the instrument is not applicable to some highly selective samples because nearly homogeneous energy is too little changed in spectral composition by passage through the wedges. This colorimeter has been used in setting up a color standard for ruby mica (*49*); for inspection of working standards, transparent and opaque, for conformity to a master standard; and for general colorimetry by difference, for both fluorescent and nonfluorescent specimens (*95*).

9·19 Photoelectric tristimulus colorimeters. If three photocells could be adjusted, as by glass filters, so that their responses were proportional throughout the visible spectrum to some linear combination [as in equation (9·3) of the standard I.C.I. distribution curves (see Fig. 9·2)], then they could be used to test whether any two light beams have the same color according to equation (9·2) and could be made to yield direct measurements of tristimulus values, X, Y, Z (*27*, *33*). There have been several good attempts to build a photoelectric tristimulus colorimeter suitable for general use (*9*, *40*, *41*, *87*, *110*), but with incomplete success (*42*, *106*, *107*). Figure 9·13 shows the degree to which filters designed by Hunter (*41*) to duplicate the I.C.I. standard observer and simultaneously to adjust a projection lamp to I.C.I. il-

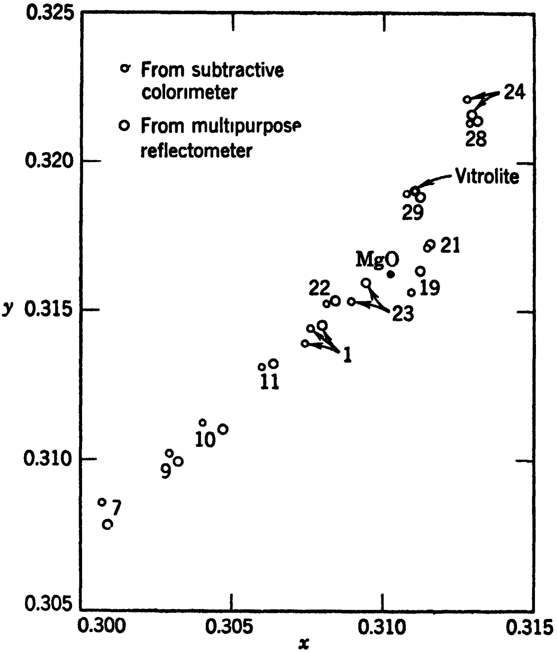


FIG. 9·15. Agreement between multipurpose reflectometer (*41*) and the chromaticity-difference (subtractive) colorimeter for near-white porcelain-enamel specimens measured relative to magnesium oxide. (Taken from *Natl. Bur. Standards Circular* C429.)

luminant C have succeeded. Figure 9·14 shows the discrepancies that the filters of his multipurpose reflectometer introduce. These discrepancies are roughly proportional to the distance from the point representing the magnesium oxide standard, and are frequently larger than 0.02 in x or y; that is, more than 10 times a reasonable chromaticity tolerance for most colorimetric work. However, for the comparison of near-white surfaces this degree of duplication is sufficient. Figure 9·15 refers to the small rectangle near the center of Fig. 9·14 and indicates that the discrepancies are less than 0.001 in x or y for comparison of near-white surfaces with magnesium oxide. In general, a similar agreement can be expected in using this photoelectric tristimulus colorimeter for the determination of small chromaticity differences between nonmetameric pairs. And even for measurement of fairly sizable nonmetameric chromaticity differences, such as analyzed spectrophotometrically in the upper portion of Fig. 9·16 (*BPB* 8/2 vs. MgO, *BG* 7/4 vs. *BG* 6/4), and small chromaticity differences with a moderate metameric component, such as shown in the lower left portion of Fig. 9·16 (Y_1 vs.

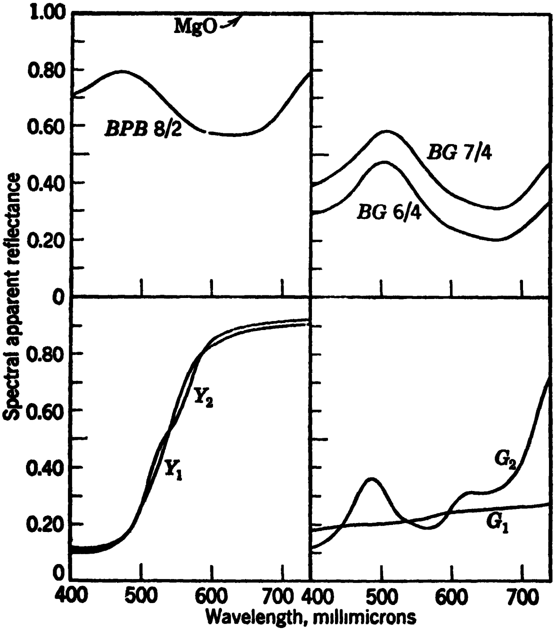


FIG. 9·16. Spectral reflectances of pairs of samples exhibiting various degrees of metamerism. Upper two pairs differ considerably in color, but show little or no metamerism. Lower two pairs are near matches; left, moderately metameric; right, strongly. (Taken from *Natl. Bur. Standards Circular* C429.)

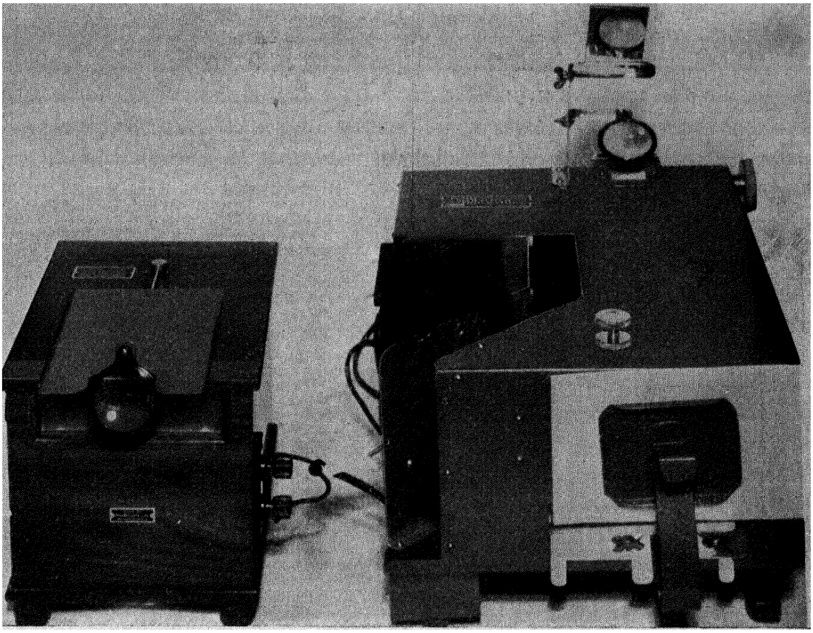


FIG. 9·17. General view of factory-control reflectometer and galvanometer. A sample of paper is in position for measurement. (Courtesy of Henry A. Gardner Laboratory.)

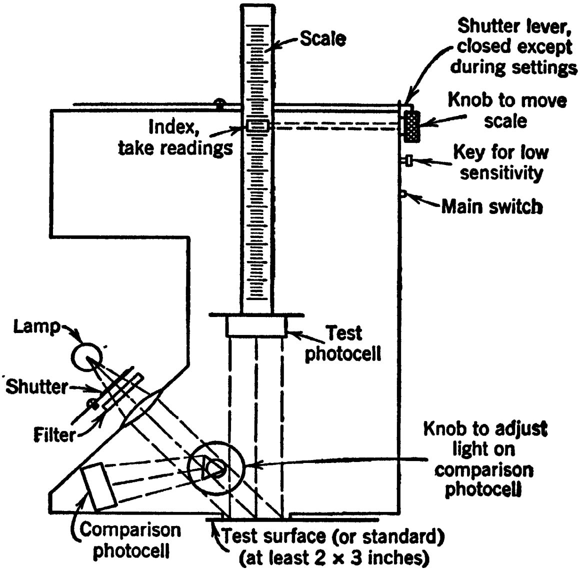

Scale
Shutter lever, closed except during settings
Knob to move scale
Index, take readings
Key for low sensitivity
Main switch
Lamp
Test photocell
Shutter
Filter
Knob to adjust light on comparison photocell
Comparison photocell
Test surface (or standard) (at least 2 × 3 inches)

FIG. 9·18. Diagram of the Hunter factory-control reflectometer, showing the arrangement of parts. A small mirror takes light from the center of the main beam to illuminate the comparison photocell. (Courtesy of Henry A. Gardner Laboratory.)

Y_2), the discrepancy is in the neighborhood of 0.002 in x or y, which is negligible for many purposes. However, for highly metameric pairs, such as shown in the lower right portion of Fig. 9·16, the discrepancy may be expected to be in the neighborhood of 0.02, just as it is for large chromaticity differences.

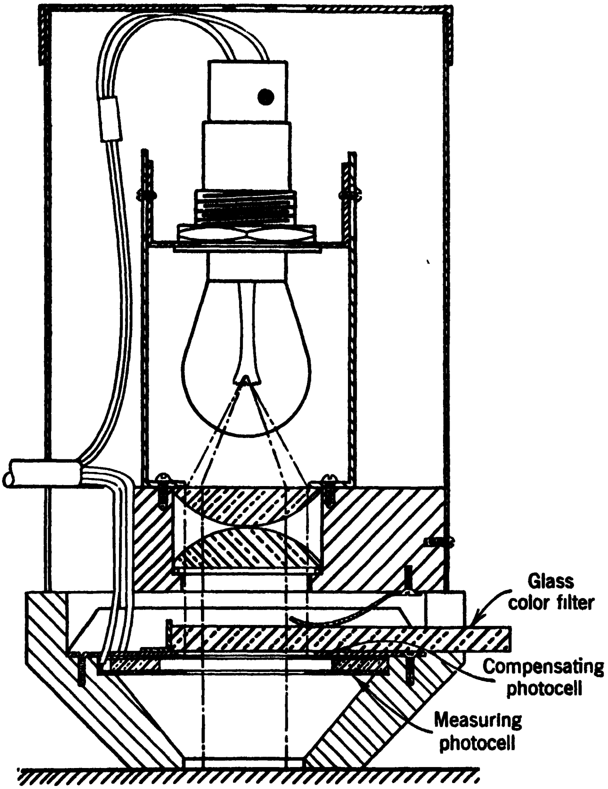


FIG. 9·19. Photoelectric reflection meter. The control box with current-measuring equipment is not shown. (Courtesy of the Photovolt Corporation.)

If the limitations of photoelectric tristimulus colorimetry are appreciated, the method is most useful in product-control colorimetry of nonfluorescent specimens by difference from a working standard. The precision of the method is comparable, though perhaps not quite equal, to the best that can be done by eye. No unusual qualifications or extended special training is required by the operator; and, compared to visual colorimetry or to indirect colorimetry by the spectrophotometer, the results are obtained very rapidly.

Figure 9·17 is a photograph of a photoelectric tristimulus reflectometer using the Hunter filters, and Fig. 9·18 is a diagram of it. Figure 9·19 is a diagram of the optical portion of another reflectometer using the Hunter filters. Both these reflectometers are commercially available. The filters are also available separately and have been used for the comparison of solutions, petroleum products, and glasses in the Lumetron photoelectric colorimeter.

D·COLOR SPECIFICATION BY MATERIAL STANDARDS

9·20 General principle. Because of the convenience of material standards of color, they are often used in commerce in preference to specification according to the more fundamental I.C.I. system. Material standards may be carried from place to place, and, if the colors are sufficiently closely spaced in the neighborhood of the unknown color, the nearest match may be found by visual comparison. The color specification consists of identifying the particular member of the system yielding a match for the color to be specified. We deal here both with systems of material standards of such scope that a considerable fraction of the colors possible in non-self-luminous objects are represented and with a few special small groups of material standards for particular purposes.

1·TRANSPARENT MEDIA

Color systems based upon transparent media take advantage of the fact that it is possible with a fixed illuminant to control the color of the transmitted light over a wide range by introducing varying amounts of three absorbing materials. This is done by permitting the light to pass through two or more elements of the absorbing medium instead of through a single element, and is called subtractive combination or mixture because the action of each element is to subtract a certain fraction of each part of the spectrum of the incident light. The color specification consists of the number of unit elements of each of the three absorbing components required to produce the color match.

9·21 Lovibond glasses. The Lovibond color system consists of three sets of colored glasses, red, yellow, and blue (*57, 58, 59*), the principal coloring materials being gold, silver, and cobalt, respectively. The unit of the scale defined by each set is arbitrary, but the three units are related by being adjusted so that, for observation by daylight, subtractive combination of one unit of each of the red, the yellow, and the blue scales results in a filter perceived as neutral or achromatic. Each scale is exemplified by many glasses, each glass being marked with the number of unit glasses to which it is equivalent. Although the original purpose of the Lovibond color system was to aid in the color control of beer, these glasses are widely used today for other products such as vegetable oils, lubricating oils, and paint vehicles.

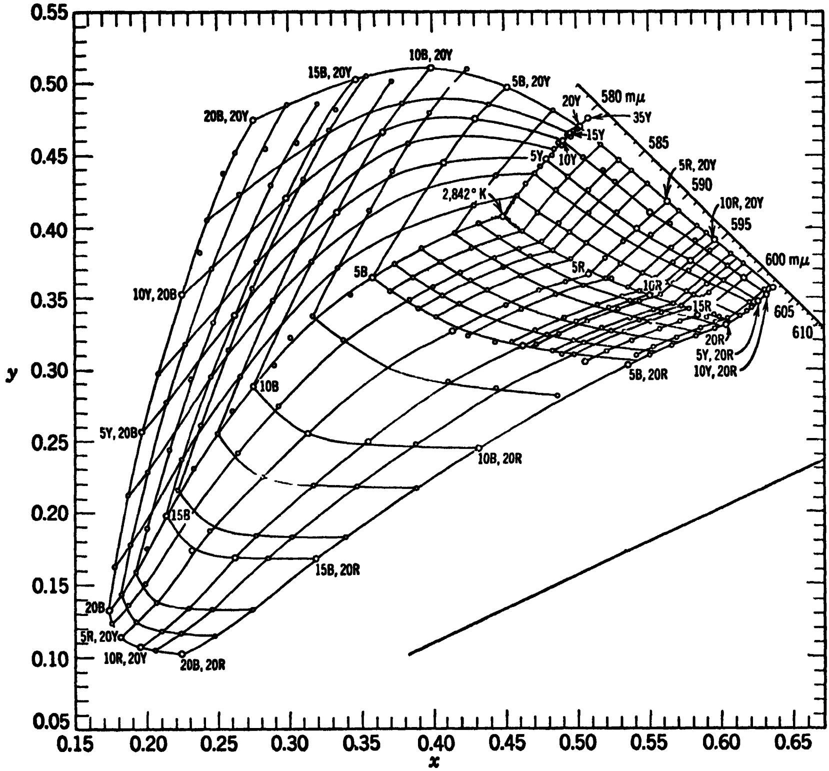


FIG. 9·20. Chromaticities of Lovibond glass combinations for illuminant *A*. (Photometry and Colorimetry Section, National Bureau of Standards.)

A spectrophotometric analysis of the Lovibond color system was made by Gibson and Harris (*25*), and a scale of the red glasses used in combination with the 35-yellow glass has been constructed by Priest and Gibson (*26*, *37*, *109*), having the same unit as the original Lovibond red scale but embodying a closer approach to the principle that the Lovibond numeral should indicate the number of unit glasses to which the single glass bearing the numeral is equivalent. Red glasses are calibrated in terms of the Priest-Gibson scale at the National Bureau of Standards and at the Electrical Testing Laboratories. The latter will also adjust the glasses to slightly lower numerals, if desired, by reducing their thickness slightly (*22*). Tintometer, Ltd., makers of the Lovibond glasses, have computed from the published spectrophotometric measurements (*25*) the chromaticity coordinates, x, y, of all the colors of the Lovibond system (*99*) produced by illuminating the glasses with I.C.I. standard illuminant B. Computation of these colors for illuminants A and C has been done at the National Bureau of Standards (*37a*). Figure 9·20 shows some preliminary results for illuminant A.

9·22 Arny solutions. The Arny solutions consist of groups of solutions whose concentrations are adjusted to produce the color match. The required concentrations are the specifications of the color. The most used group is a triad consisting of half-normal aqueous solutions of cobalt chloride (red), ferric chloride (yellow), and copper sulfate (blue) in 1 per cent hydrochloric acid. This group produces all colors except deep blue and deep red; it is supplemented by a triad of ammoniated aqueous solutions of cobalt, chromium, and copper salts, and by a pair consisting of aqueous solutions of potassium permanganate and potassium dichromate (*6*, *7*, *8*). Mellon and Martin (*68*) have reported the spectral transmittancies for a number of solutions for colorimetric standards, including the Arny solutions at three or four concentrations, each for the spectral range 440 to 700 mμ. By extrapolation of these data it is possible to find approximately the tristimulus values and chromaticity coordinates, x, y, on the standard coordinate system adopted in 1931 for these solutions, just as was done by Mellon (*69*) for the coordinate system used in America before the international agreement. In this way it is possible to transform color specifications from the standard system into the required concentrations of the Arny solutions, and the reverse. The Arny solutions are used in the eleventh edition of the United States Pharmacopoeia as standards for the color of cod-liver oil and in carbonization tests with sulfuric acid for twenty-eight organic compounds.

9·23 One-dimensional color scales. There are many tests analogous to the comparison of a solution of an unknown amount of a constituent with a series of suitably prepared standard solutions to find the concentration of the specimen. In these tests the colors of the unknowns exhibit a one-dimensional change with concentration; and, although this change may be complicated in terms of luminous transmittance and chromaticity coordinates (*69*), a suitably spaced series of standards over this range of colors will yield the desired concentration either by actual match with one of the standards or by visual interpolation among them. Such a series of standards is said to constitute a *color scale*. The ideal material from which to make the standards is the constituent of the unknown itself; in this way there is guaranteed not only a perfect color match at some point along the scale, but also a perfectly nonmetameric match so that variation of the illuminant or individual-observer variations are generally unimportant.

However, if the unknown is impermanent, it may become necessary to try to duplicate the desired colors in a more nearly permanent medium. Glass is a frequent choice because of its generally superior permanence. Some degree of metamerism has then to be tolerated because the standards have coloring constituents not a perfect spectral match for the unknown. It is also rare that a perfect job of color matching for any standard illuminant and observer is done. The observer is then faced with what is often a difficult, and sometimes an impossible, task.* He must estimate the position of the unknown color on the scale, and oftentimes it will seem to him that the unknown color is not equal to any of the standard colors nor intermediate between any two of them. The concepts in terms of which the observer perceives these color differences then come into play. If he judges the color difference between the two luminous areas presented to him in terms of hue, brightness, and saturation, as is fairly common, he could estimate the position of the unknown on the color scale as the point on the scale yielding the same hue, or as that yielding the same brightness or the same saturation; or he could try to estimate the point on the scale yielding the closest color match; or he could disregard brightness differences and try to estimate the point on the scale yielding the closest chromaticity match. The determination becomes an estimate based on what criterion of equivalence is used by the observer, and it depends upon his mental furniture in an essentially indescribable way. In spite of these drawbacks, a good color scale is a useful time-saver, as long as it is not used in attempts to provide a one-dimensional solution to what is essentially a multidimensional problem.

Judgments of position on the color scale according to equality of brightness can be expected to correspond to luminous transmittance. Judgments according to equality of hue agree well with the Munsell renotation hue (to be discussed presently); loci of constant hue for ordinary conditions of observation are indicated on the (x, y) diagram by the curved lines of Fig. 9·3 separating the areas corresponding to the various hue names. Judgments according to equality of saturation agree well with Munsell renotation chroma (see Fig. 9·33). If there is only secondary brightness variation along the scale, judgments of nearest chromaticity match may be found approximately by taking the shortest distance on a uniform-chromaticity-scale diagram (*11, 18, 41, 46, 100, 101*). Figure 9·21 shows the uniform-chromaticity-scale triangle according to Judd (*46*). If the chromaticity coordinates of a color are (x, y) in the standard I.C.I. system, the color would have chromaticity co-

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* See Chapter 3, Section 3·11, for a discussion of the use of such permanent standards in comparators.

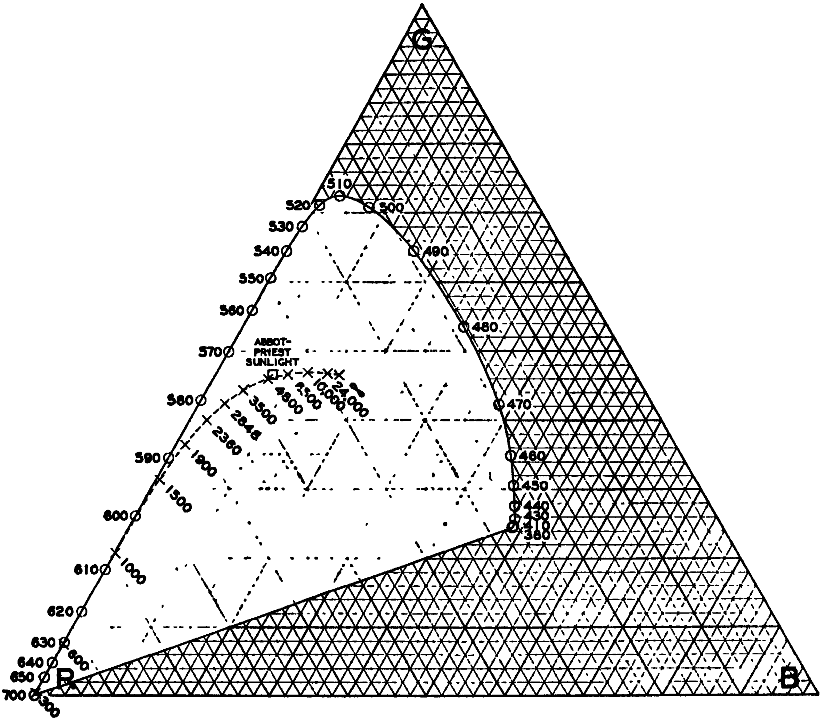

FIG. 9·21. Uniform-chromaticity-scale triangle according to Judd (*46*). The length of the straight line connecting the points representing any two chromaticities on this triangle is approximately proportional to the perceptibility of the chromaticity difference.

ordinates (r, g) in this uniform-chromaticity-scale triangle in accord with equation (9·5):

$$r = \frac{2.7760x + 2.1543y - 0.1192}{-1.0000x + 6.3553y + 1.5405}$$
$$g = \frac{-2.9446x + 5.0323y + 0.8283}{-1.0000x + 6.3553y + 1.5405} \qquad (9\cdot5)$$

On this diagram the ellipses of Fig. 9·6 would be equal tangent circles. If there is primary variation of both luminance and chromaticity, no reliable way of estimating the nearest color match has yet been developed. According to the O.S.A. Colorimetry Committee (*19*), "The complete experimental clarification of this problem is one of the major programs yet to be undertaken in the field of colorimetric research."

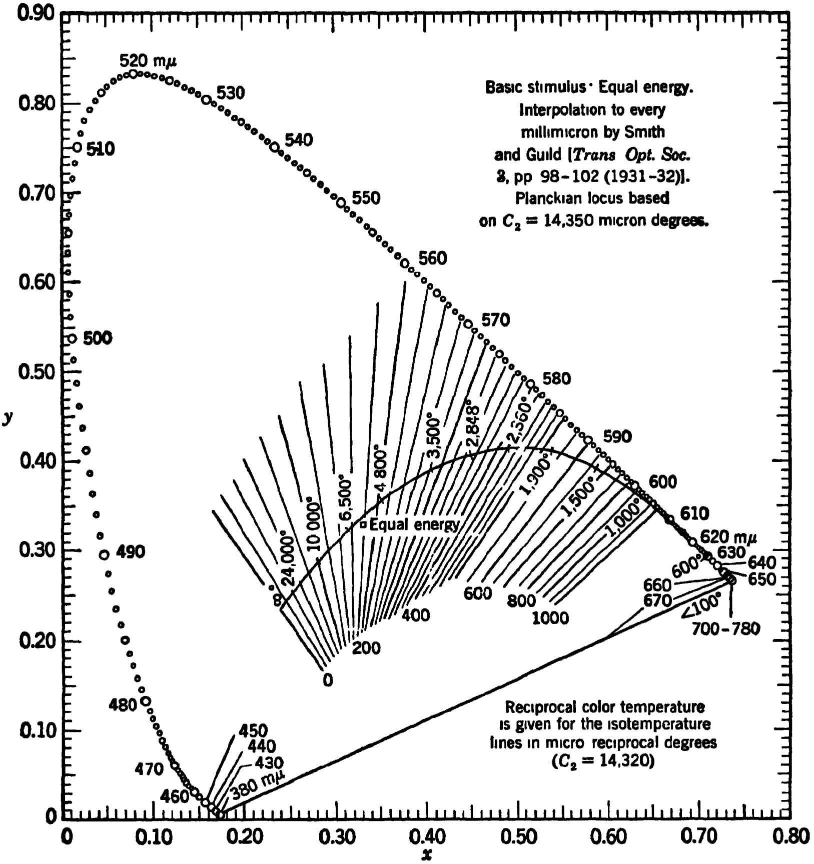

Fig. 9·22. Isotemperature lines for evaluation of a correlated color temperature for non-Planckian colors. On Fig. 9·21 these lines are normal to the Planckian locus. (Colorimetry Section, National Bureau of Standards.)

9·24 Color temperature. Perhaps the most widely used one-dimensional color scale is that of color temperature for classifying light sources. The *color temperature* of a light source is the temperature at which the walls of a furnace must be maintained so that light from a small hole in it shall yield the chromaticity of the source to be specified. The color scale thus consists of the series of lights producible by closed-cavity radiation and is specified by temperature on the absolute scale (degrees Kelvin). Working standards of color temperature may consist of an incandescent lamp operating at a fixed voltage combined with a series of amber or blue glasses, like the Lovibond blue glasses; but by far the most common way of producing these chromaticities over moderate ranges of color temperature is by variation of the voltage applied to an incandescent lamp. The locus of these chromaticities (the so-called Planckian locus) is shown on Figs. 9·5 and 9·21. If the chromaticity of the light source is close to, but not exactly equal to, any of the Planckian chromaticities, still it is possible to correlate a color temperature with the source by taking the nearest chromaticity match. Figure 9·22 shows this correlation (*47*). The isotemperature lines, which cut the Planckian locus at varying angles, are all such as to be perpendicular to this curve on Fig. 9·21. Since color temperature specifies only the chromaticity of a light, there are many spectral compositions corresponding to the same color temperature. Color temperature of an illuminant is therefore an incomplete and unreliable indication of the rendering of the colors of objects illuminated by it or of the photographic effect of the illuminant. To make color temperature a meaningful and useful basis for comparing two lights it must also be known that they are spectrally similar; thus, incandescent lamps may be usefully compared by means of color temperature, and fluorescent lamps with about the same admixture of mercury spectrum may also be so intercompared, but not incandescent lamps with fluorescent.

9·25 Lovibond grading of vegetable oils. The Lovibond glasses are used extensively in the grading of vegetable oils in this country. McNicholas (*67*) has shown, from the spectral transmittance of representative samples of refined cottonseed oils and oil of sesame, peanut, soybean, corn, rape, and olives, that these oils owe their colors to independently varying amounts of two groups of coloring matters commonly found in plants, a group of brown pigments and a group of green (chlorophyll compounds). For commercial purposes the colors of these oils are graded by the number of Lovibond red units required in combination with Lovibond 35-yellow to color match a 5¼-inch layer of the oil. Figure 9·23 shows (*67*) the spectral transmittance of a group of cottonseed oils (solid curves) and other vegetable oils (dotted curves) all of which have color grades between 8.0 and 10.0 Lovibond red units on the 35-yellow plus red scale. Figure 9·23 also shows the spectral transmittance of these limiting Lovibond glass combinations (solid curves with solid circles). It will be noted first that the oils all make metameric pairs with the Lovibond glass combinations. A standard light source is thus required, and a certain amount of individual observer difference may be expected in grading oils by this method. Since there are two main groups of pigments in the oils, it is also to be expected that no one-dimensional grading system, like the scale of 35-yellow plus some amount of red, will yield color matches. Computation of the tristimulus values from the spectral-transmittance curves and plotting on

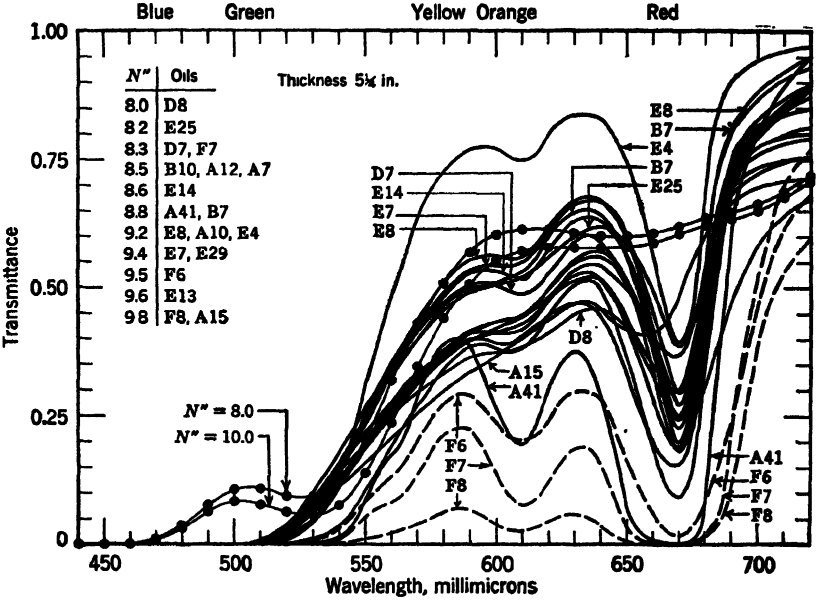

Fig. 9·23. Spectral transmittance of vegetable oils compared to that of the Lovibond glass combinations (*solid dots*) giving the nearest chromaticity match. [Taken from research paper (*67*) by McNicholas.]

a Maxwell triangle (see Fig. 9·24 due to McNicholas) reveal this spreading of the chromaticity points over an area instead of along a single line and show further that the Lovibond 35Y plus R locus is too far from the spectrum locus to be a good match for the center of the area except for Lovibond red less than 3 units. There is considerable doubt, therefore, what Lovibond grade corresponds to a given spectral-transmittance curve of Fig. 9·23. McNicholas chose to base the decision on nearest chromaticity match and made use of the uniform-chromaticity-scale diagram shown in Fig. 9·21. The short straight lines on Fig. 9·24 intersecting Lovibond 35Y plus R locus at somewhat oblique angles would be perpendicular to the corresponding locus on Fig. 9·21. In spite of these difficulties the method is well suited to indicate the quality and salability of vegetable oils, and it has been used for more than 30 years. Because of the difficulties, however, and the further disadvantage of being dependent upon a foreign source of glass standards, there have been fairly continuous agitation and research work intended to lead to another, perhaps photoelectric, method. But the Lovibond color scale, 35Y plus R, is so ingrained in the purchase and sale of vegetable oil that it may well be that a substitute method will still have to give results expressed on that scale.

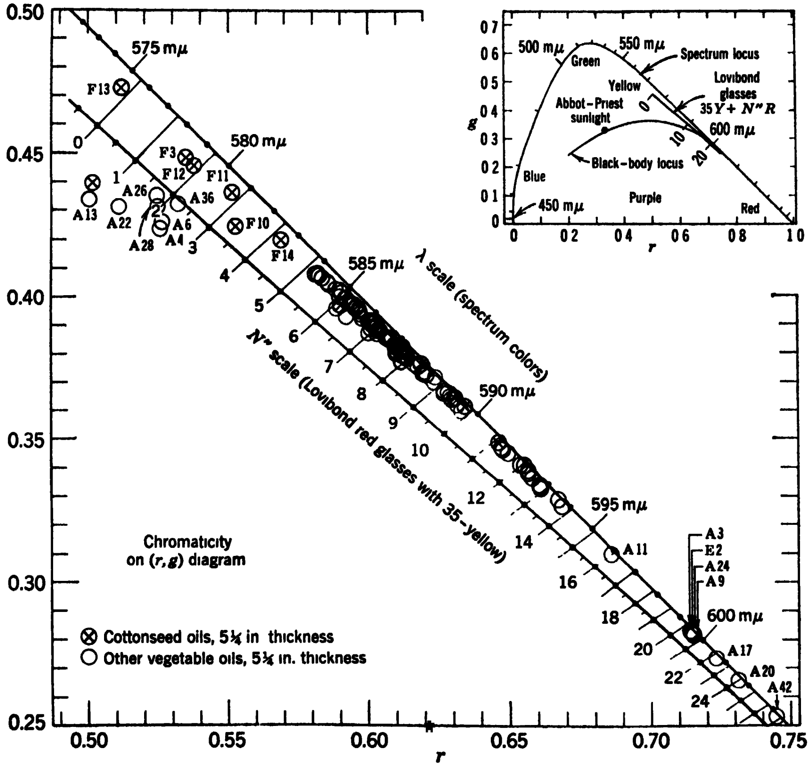

FIG. 9·24. Chromaticities of vegetable oils compared to those of the Lovibond glass combinations used in grading them. [Taken from research paper (*67*) by McNicholas.]

9·26 Grading of lubricating oils by Union colorimeter. For more than 20 years the color of lubricating oils and petrolatum has been graded by comparison with the colors of 12 glass standards (*4*). The petroleum product in a 33-mm. layer and the standard are illuminated by artificial daylight produced by combining an incandescent lamp of color temperature approximately 2750°K. with a filter of Corning Daylite glass specially selected to have spectral transmittances within specified tolerances and further to have for standard illuminant A luminous transmittance, T_w, and chromaticity coordinates, x, y, z, within the limits:

T_w	0.107 to 0.160
x	.314 to .330
y	.337 to .341
z	.329 to .349

The specimen holder, the magazine containing the glass color standards, the artificial daylight assembly, and a viewing diaphragm defining the direction of view are mounted together to form a portable instrument known as the Union colorimeter. Figure 9·25 is a photograph of the Union colorimeter.

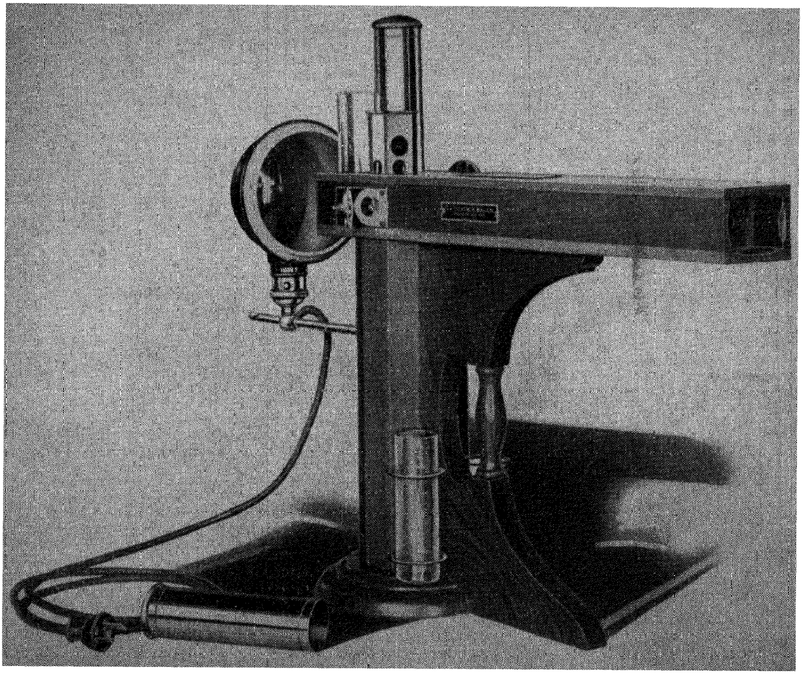

FIG. 9·25. General view of the Union colorimeter. (Courtesy of the Tagliabue Company.)

Table 9·10 gives the Lovibond analysis of the glass color standards (*4*), the luminous transmittance, T_w, and chromaticity coordinates, x, y, for illuminant C (*100*), and the color names used by the National Petroleum Association. Figure 9·26 shows by large circles on the (x, y) diagram the chromaticities of these glass color standards compared to those of 22 petroleum products (small circles) measured by Diller, De Gray, and Wilson (*20*). It will be noted from Fig. 9·26 that the Union colorimeter glasses yield good chromaticity matches for the petroleum products represented except for A.S.T.M. color numbers 1½ to 3½, in which range the oil points fall between the spectrum locus and the Union locus. Note also that the oil points and the Union locus above A.S.T.M. color number 5 fall on the spectrum locus; that is, the standard observer would find that each such glass standard and each such oil color matches some part of the spectrum between 605 and 640 mμ. It is possible that some improvement could be achieved by choosing glass standards of higher excitation purity for the color numbers 2, 2½, 3, and 3½; such a change would make it easier for an observer to decide which glass standard is closest in chromaticity to such oils as are represented on Fig. 9·26. However, the difficulty that has probably prevented the Union colorimeter from becoming the basis of a standard method is that the luminous transmittances of lubricating oils fail to correlate well with their chromaticities. This essential difficulty would not be overcome by any choice of standards in a one-dimensional series. Note from Table 9·10 that glasses 6 and 7 have nearly the same chromaticity but differ by more than a factor of 2 in luminous transmittance. If, as seems probable from the words *lighter* and *darker* used in describing

TABLE 9·10 GLASS STANDARDS OF THE A.S.T.M. UNION COLORIMETER

A.S.T.M. Color Numbers	National Petroleum Association Color Numbers	Lovibond Analysis			I.C I. Specification			National Petroleum Association Names
		Red	Yellow	Blue	Luminous Transmittance, (*T*)	Chromaticity Coordinates		
						x	*y*	
1	1	0.12	2.4		0.751	0.349	0.382	Lily white
1½	1½	0.60	8.0	. . .	.654	.400	.446	Cream white
2	2	2.5	26.0	. . .	.443	.472	.476	Extra pale
2½	2½	4.6	27.0	. . . .	.365	.498	.457	Extra lemon pale
3	3	6.9	32.0	. . . .	.287	.525	.440	Lemon pale
3½	3½	9.4	45.0	. . .	.211	.556	.423	Extra orange pale
4	4	14.0	50.0	0.55	.096	.591	.400	Orange pale
4½	4½	21.0	56.0	0.55	.065	.620	.376	Pale
5	5	35.0	93.0	. . .	.036	.653	.347	Light red
6	6	60.0	60 0	0.55	.017	.676	.323	Dark red
7	7	60.0	106.0	1.8	.0066	.684	.316	Claret red
8	8	166.0	64.0	. . .	.0020	.714	.286	. ...

the method (*4*) and from a proposed photoelectric method of test (*2*), the chief intent is to specify the lightness or darkness of the oil color, it would be more helpful to bring together the photometric fields to be compared by a suitable prism eyepiece so that brightness differences could be more readily detected; or even use a photometer such as the Martens (Fig. 9·10) to determine by comparison with the glass standards the luminous transmittance of the oil. The Union colorimeter, however, in spite of occasional important ambiguity of setting, is very useful, and the Union color scale is well known in the petroleum industry.

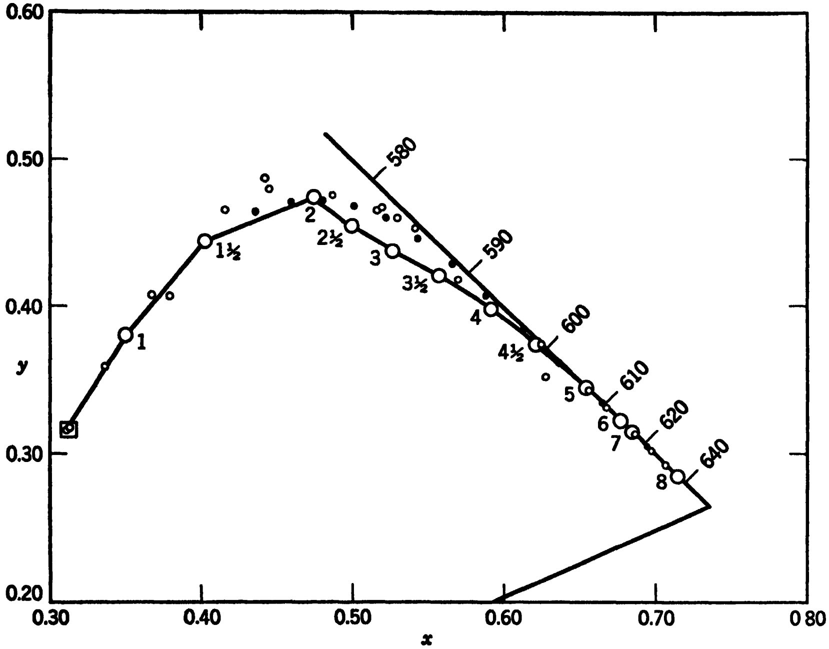

Fig. 9·26. Chromaticities of the glass color standards of the Union colorimeter (*large circles*) compared to those of petroleum products (*small circles*) (*20*) and to those of the United States color standards for rosin (*13*) (*solid circles*). Wavelengths of the long-wave portion of the spectrum are indicated in millimicrons.

9·27 Grading of refined petroleum oil by Saybolt chromometer. The grading of naphthas, kerosenes, and so on, has for many years been carried out by comparison of the color of rather thick layers (up to 20 inches) of the refined oil with the colors of a set of three color standards made of yellowish glass. The Saybolt chromometer is a device for carrying out this comparison. It consists of an artificial daylight lamp meeting the same requirements as given above for the

A.S.T.M. Union colorimeter, a graduated tube for the oil specimen, a holder for the glass color standards, and a prism eyepiece to bring into juxtaposition the two fields to be compared. Figure 9·27 is a photograph of the Saybolt chromometer. There is an open ungraduated tube beneath the holder for the glass standards that serves to duplicate to a degree on the standard side of the instrument the effect of light multiply reflected within the specimen tube. The glass standards consist of two whole discs and one "one-half" disc; these standards must have luminous transmittances, T_w, and chromaticity coordinates, x, y, for illuminant C, as follows:

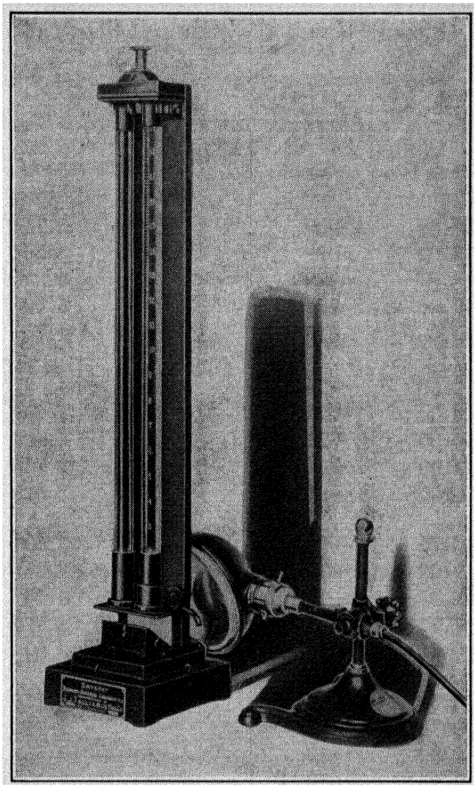

FIG. 9·27. General view of the Saybolt chromometer. (Courtesy of the Tagliabue Company.)

	WHOLE DISCS	ONE-HALF DISCS
T_w	0.860 to 0.865	0.888 to 0.891
x	.342 to .350	.327 to .331
y	.367 to .378	.344 to .350
z	.272 to .291	.319 to .330

Oils having colors closely resembling that of distilled water are graded on the Saybolt chromometer by comparison with the half disc; more yellowish oils by one or two whole discs. The depth of oil yielding the closest color match with the glass disc is found by a prescribed procedure (*3*), and the color of the oil specimen is designated by a number defined from the disc used and the required depth of oil. Table 9·11 gives this definition (*3*).

Table 9·11 Saybolt Chromometer Color Corresponding to Depths of Oil

Number of Discs	*Depth of Oil, Inches*	*Color*	*Number of Discs*	*Depth of Oil, Inches*	*Color*
½	20.00	+30	2	5.75	5
½	18.00	+29	2	5.50	4
½	16.00	+28	2	5.25	3
½	14.00	+27	2	5.00	2
½	12.00	+26	2	4.75	1
1	20.00	+25	2	4.50	0
1	18.00	+24	2	4.25	−1
1	16.00	+23	2	4.00	−2
1	14.00	+22	2	3.75	−3
1	12.00	+21	2	3.625	−4
1	10.75	+20	2	3.50	−5
1	9.50	+19	2	3.375	−6
1	8.25	+18	2	3.25	−7
1	7.25	+17	2	3.125	−8
1	6.25	+16	2	3.00	−9
2	10.50	+15	2	2.875	−10
2	9.75	+14	2	2.75	−11
2	9.00	+13	2	2.625	−12
2	8.25	+12	2	2.50	−13
2	7.75	+11	2	2.375	−14
2	7.25	+10	2	2.25	−15
2	6.75	+9	2	2.125	−16
2	6.50	+8			
2	6.25	+7			
2	6.00	+6			

In spite of the fact that the prism eyepiece brings the fields to be compared into juxtaposition and so facilitates the detection of differences in brightness, the change in luminous transmittance with thickness of these refined oils is so slight that it is much less easily detected than the corresponding chromaticity change. The settings of the Saybolt chromometer therefore probably depend essentially on nearest chromaticity match. If the oil sample is turbid, however, not even an approximate match can be obtained, and the method may be inapplicable. In these cases a thickness of the specimen yielding a chromaticity match is much darker than the standard, and no reliable setting of depth of sample yielding nearest match can be found.

Figure 9·28 shows a portion of the (x, y) chromaticity diagram on which have been plotted the rectangles corresponding to the chromaticity tolerances for Saybolt half discs and whole discs (see above). It will be noted that the tolerances are fairly wide so that variations of ±10 per cent of the chromaticity change caused by introduction of the disc into the daylight beam are permissible. From Table 9·11 it may be seen that a whole disc is never equivalent to as much as a difference of 10 in Saybolt color; so the tolerances are equivalent to less than 1 in Saybolt color. On Fig. 9·28 is shown by a circle the point representing Union color No. 1. It is of interest to inquire what would be the Saybolt color of an oil having A.S.T.M. color No. 1, because we could then write down an approximate interconversion of the two scales. Since the Saybolt chromometer makes use of variable depths of oil specimen, we must first find out how the chromaticity of an oil specimen on the (x, y) diagram varies with its thickness. An approximate answer is

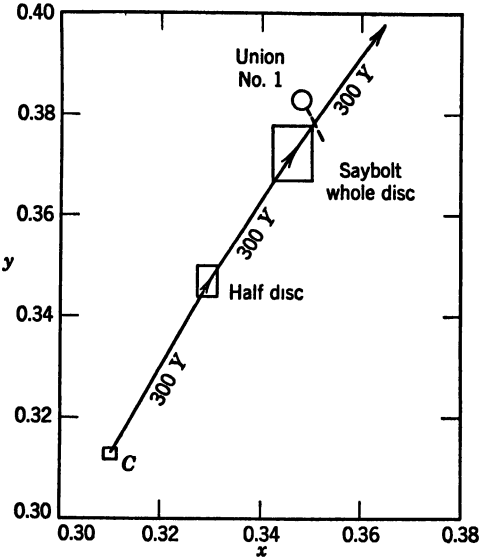

FIG. 9·28. Chromaticities of the Saybolt color standards compared to that for Union No. 1 color, and to those of the yellowish wedge of the chromaticity-difference colorimeter (*arrows*).

supplied by the calibration of the yellowish wedge of the chromaticity-difference colorimeter (Fig. 9·12). The spectral transmittances of this wedge resemble closely those of refined petroleum oils. Figure 9·28 shows three vectors each corresponding to 300 yellow units on the chromaticity-difference colorimeter. The first vector starts at the point representing illuminant C, and it is seen that a difference of 300 yellow units is equivalent within the prescribed tolerances to the Saybolt half disc. The second vector starts at the end of the first and indicates that adding 300 additional yellow units bridges the chromaticity difference between the Saybolt half disc and the Saybolt whole disc. The third vector commences at the end of the second and shows that increasing thickness of a medium like refined petroleum oil gives a slightly curving line on the (x, y) diagram with a decreasing scale as thickness is increased. The point representing Union color No. 1 does not fall exactly on this line, but the dotted line drawn from this point intersecting the third vector corresponds to the perpendicular to the vector plotted on a uniform-chromaticity-scale triangle (Fig. 9·21) and indicates the closest chromaticity match. The direction of the dotted line may also be found with good approximation from the ellipses of Fig. 9·6; it is the direction of the line connecting the center of the ellipse with the point of tangency to the vector. From the intersection of the dotted line with the vector it may be seen that the chromaticity of Union color No. 1 is the same as that of about 1.10 Saybolt disc. An oil of Union color No. 1 will therefore normally match 1.00 Saybolt disc in a thickness of $33.0/1.10 = 30.0$ mm., and it will match two whole discs in a thickness of 60.0 mm. or 2.365 inches. Reference to Table 9·11 shows that such an oil would have Saybolt color equal to -14; this corresponds nearly to the yellowest oil indicated on the Saybolt scale. Thus, the Saybolt scale serves to fractionate the first unit of the Union colorimeter scale.

If we are permitted to evaluate colors on the Union scale in accord with the fraction, f, of the depth of a standard solution such that unit depth of a standard solution matches Union color No. 1, then we may find the Union color, f, corresponding to any Saybolt number from the number, n, of whole discs used and the depth, D, defining the Saybolt number (Table 9·11) by the relation,

$$f = \frac{2.365n}{2D} = \frac{1.182n}{D} \tag{9·6}$$

Table 9·12 shows the Union color, f, corresponding to each Saybolt color in accord with equation (9·6). Correlations obtained from actual comparison of specimens to the two sets of standards may differ somewhat from that indicated in Table 9·12 if the standard solution used to fractionate the first step of the Union color scale has markedly different spectral transmittance from the yellowish wedge of the chromaticity-difference colorimeter (Fig. 9·12), or if the criterion of nearest chromaticity match be not followed exactly.

Table 9·12 Union Color, f, Corresponding to Saybolt Color Number from Equation (9·6)

Saybolt Number	*Union Color, (f)*	*Saybolt Number*	*Union Color, (f)*	*Saybolt Number*	*Union Color, (f)*
+30	0.030	+15	0.225	0	0.526
+29	.033	+14	.243	−1	.557
+28	.037	+13	.262	−2	.592
+27	.042	+12	.286	−3	.631
+26	.049	+11	.305	−4	.652
+25	.059	+10	.326	−5	.677
+24	.066	+9	.350	−6	.701
+23	.074	+8	.364	−7	.727
+22	.084	+7	.376	−8	.758
+21	.098	+6	.393	−9	.789
+20	.110	+5	.411	−10	.823
+19	.124	+4	.430	−11	.860
+18	.143	+3	.450	−12	.901
+17	.163	+2	.474	−13	.947
+16	.189	+1	.498	−14	.996
				−15	1.05
				−16	1.11

9·28 United States color standards for rosin. Gum rosin has been graded by color for more than 50 years. Up to 1914 the color standards were made of rosin itself in spite of the relative impermanence of its color, and from 1914 to 1936 standards composed of combinations of Lovibond glasses were used. Bryce (*13*) has described the selection of the present twelve official standards composed of two components of colored glass combined with one component of clear glass, all three cemented together with Canada balsam. The various combinations are given letter designations denoting the grades of rosin delimited by them and have legal status under the Naval Stores Act. The cemented face of the clear glass in each combination is fine ground so as to duplicate the slight turbidity characteristic of molded samples of rosin, which commonly contain traces of fine dirt. The chromaticities are shown by solid circles on Fig. 9·26, and it will be noted that they cover approximately the same chromaticity range as A.S.T.M. Union color 1½ to 8. The chromaticity spacing was adjusted by means of the uniform-chromaticity-scale triangle of Fig. 9·21 so as to progress regularly from small steps for yellow rosins to steps of about four times the initial size for reddish orange rosins.*

9·29 Color standards for paint vehicles. Many special color scales have been set up for the specification of paint vehicles (varnishes, linseed oil, tung oil, and so on). A solution of nickel sulfate and iodine (*12*) is used to define the darkest color permissible for spar varnish. A color comparator having eighteen glass color standards made by Hellige, Inc., has been used for similar purposes. The Pfund color grader, made by Munsell Color Co., compares a variable thickness of the unknown specimen with a variable thickness of an amber colored glass (*89*). The standard is wedge shaped, and the cell for the specimen is likewise wedge shaped. The Parlin (or Cargille) color standards consist of a set of thirty-five solutions. The first ten are Hazen platinum-cobalt solutions (*38*), developed originally to measure the color of natural waters and still used for that purpose under the name of A.P.H.A. (American Public Health Association) standards (*1a*). The remainder of the Parlin color standards are caramel solutions. They have been adopted tentatively by the A.S.T.M. (Designation D365-39T) for testing the color of soluble nitrocellulose base solutions. The Pratt and Lambert color standards are varnish mixtures calibrated against the Pfund color grader. The DuPont colorimeter employs six glass plates as color standards, together with a wedge of the same glass permitting a continuous variation of color between the standards. The Gardner color standards consist of eighteen combinations of the red and yellow Arny solutions. Gardner has determined the Arny and Lovibond specification for the nearest matches for all the above-mentioned sets of color standards and has also obtained the nearest equivalents in terms of potassium dichromate solutions (*24*). From these nearest equivalents it is possible to express color specifications given by any of these means in terms of any other of them.

The British Paint Research Station has recommended (*105*) combinations of Lovibond glasses for color-grading oils and varnishes. Some of the combinations involve colorless or blue glasses to be combined with the oil to match red and yellow glasses, and a device facilitating the setting up of such combinations is also recommended. The Lovibond glasses are mounted in a slide, and the two photometric fields to be compared are brought into juxtaposition by mirrors.

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* Osborn and Kenyon have studied rosin colors spectrophotometrically. See *J. Optical Soc. Am.*, **38**, 660 (1948) and *Ind. Eng. Chem.*, *Anal. Ed.*, **18**, 523 (1946).

2·PIGMENTED OR DYED SURFACES

If the material standards are pigmented or dyed surfaces, no automatically convenient notation, such as suggested by additive combination of lights or subtractive combination of absorbing elements, is available. Any systematic aspect of the color specification must be derived from the method of identifying the various members of the set of colored surfaces serving as standards.

9·30 Color dictionaries. From a color dictionary are obtained definitions of color names in terms of material standards. The primary aim is therefore to provide an array of named surface colors adequate for the purpose, and any arrangement or organization of the colors serves only the secondary purpose of assisting the user to find the one which most nearly matches.

The Maerz and Paul *Dictionary of Color* (*64*) is the foremost authority on color names. It contains about 7000 different samples of color printed on semiglossy paper, and there are listed about 4000 color names which are keyed to one or another of the color samples. These names are drawn from usage in many fields: paint, textile, ceramic, scientific, technical, and artistic. The samples are also identified by plate, column, and row, and because of their large number and fairly uniform color distribution it is usually possible to find among them a sample approaching what is called a "commercial color match" for any given uniform opaque surface. On this account the Maerz and Paul *Dictionary* finds a considerable application as a collection of color standards quite separate from its primary function of defining color names. There are noticeable color differences between corresponding samples in different copies of the *Dictionary*, but the differences have been held to a reasonably small amount by discarding the less satisfactory printed sheets.

The accepted authority for color names in the textile and allied industries is the Textile Color Card Association of the United States. The T.C.C.A. has issued nine editions of a standard color card since 1915, the current edition (*104*) containing 216 color samples of pure dye silk; furthermore, the T.C.C.A. issues to its members several seasonal cards each year. All colors of these standard and seasonal cards are identified by name and cable number; the standard colors have been measured by spectrophotometric and colorimetric procedures, and luminous reflectance, Y/Y_0, relative to magnesium oxide, and chromaticity coordinates, x, y, for illuminant C have been published (*95*).

A color dictionary much used for the specification of the colors of flowers, insects, and birds was prepared in 1912 by Ridgway (*97*). This outstanding pioneer work contains about 1000 named color samples of paper painted by hand. Each chart shows columns of colors of the same dominant wavelength progressing from each chromatic color at the middle of the column toward white at the top, and toward black at the bottom; and there are five series of such columns, each one encompassing the entire hue circuit, but at different purities. Many of the names were coined at the time of publication to fill in gaps in popular color nomenclature and so have not much descriptive value. Each sample is arbitrarily identified by column, row, and series, however; and there is an alphabetical list of the color names giving this identification.

9·31 Ostwald color system. The notation of the Ostwald system is based on the properties of idealized pigment surfaces having spectral reflectance constant at a certain value between two complementary wavelengths and reflectance constant at a certain other value at other parts of the spectrum (*23*, *85*). The *full colors* are those that have the low values of spectral reflectance equal to zero and the high ones equal to 100 per cent. The difference between these two reflectances for other idealized pigment surfaces is the *full color content*, the value of the low reflectance is the *white content*, and the difference between the high reflectance and 100 per cent is the *black content*. The complete Ostwald notation consists of a number and two letters. The number indicates dominant (or complementary) wavelength on an arbitrary but approximately uniform perceptual scale, and is called *Ostwald hue number*. The first letter indicates white content, *a* being a white content of 89.13 per cent, which is as near to 100 per cent as is practicable for usual pigment-vehicle combinations, and other letters in alphabetical sequence indicating decreasing white content on a logarithmic scale. The second letter indicates black content, *a* being a black content of 10.87 per cent, which is as near to zero as is practicable, and other letters in alphabetical sequence indicating increasing black content on a logarithmic scale. The logarithmic scales were thought by Ostwald to insure uniform color scales, but this is true only to a rough approximation. Since the per cent white content, black content, and full color content must necessarily add up to 100, no explicit indication of the latter is required.

The Ostwald ideas have been a considerable aid in thinking about color relationships on the part of those who duplicate colors by mixtures of chromatic pigments with white and black pigments, and they have served as a guide in the selection of combinations of such colors to produce pleasing effects. However, the use of these idealized pigment surfaces as a basis for a system of colorimetry has been hampered by the fact that actual pigment surfaces approximate them rather poorly, and by the fact that not all actual pigment surfaces can be color matched by one of these ideal surfaces. Still, color charts made up more or less in accord with the Ostwald principles have been widely used for color standards and for the selection of harmonizing colors (*43, 84, 86, 103*). Of these the Jacobson *Color Harmony Manual* (*43*) is pre-eminent not only because of its technical excellence, but also because Foss (*23*) has given a clear statement of which of the somewhat contradictory Ostwald principles was followed in its construction, and Granville and Jacobson (*35*) have made a spectrophotometric study of the color chips and have published luminous reflectance, Y/Y_0, relative to magnesium oxide, and chromaticity coordinates, x, y, for every chip. These chips are therefore valuable for use in colorimetry by difference from a working standard (see Division C), and the fact that the chip is a member of an orderly arrangement of colors facilitates the selection of a working standard for any particular purpose.

9·32 Munsell color system. The basis of the Munsell system is description of colors perceived to belong to surfaces in terms of hue, lightness, and saturation. Each such tridimensional description can be represented by a point plotted in a space diagram known as the *surface-color solid*; see Fig. 9·29. In the surface-color solid the central axis represents the grays extending from black at the bottom to white at the top. *Lightness* of a chromatic (nongray) color determines the gray to which it is equivalent on this scale. Lightness is represented in the color solid by distance above the base plane. *Hue* determines whether a color is perceived as red, yellow, green, blue, purple, or some intermediate; it is

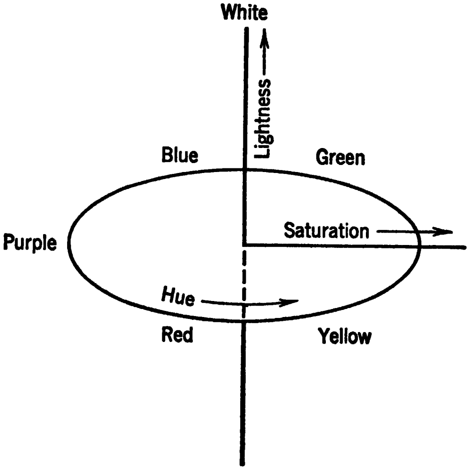


FIG. 9·29. Dimensions of the surface-color solid.

represented in the color solid by angle about the central axis. *Saturation* indicates the degree of departure of a surface-color perception from the gray of the same lightness; it is represented by distance from the central gray axis.

The Munsell color system specifies a surface color by giving for usual viewing conditions its position on more or less arbitrary hue, lightness, and saturation scales having perceptually nearly uniform steps (*72*). The Munsell term corresponding to lightness is Munsell value; that for saturation is Munsell chroma; and that for hue is Munsell hue. Munsell value is zero for the ideal black surface having luminous reflectance equal to zero, and it is 10 for the ideal white diffusing surface having luminous reflectance equal to 1. Munsell chroma is expressed in arbitrary units intended to be perceptually of the same size regardless of value and hue. The strongest known pigment colors have chromas of about 16; neutral grays have zero chroma as do black and white. Munsell hue is expressed on a scale intended to divide the hue circuit (red, yellow, green, blue, purple, back to red) into 100 perceptually equal steps. According to one convention the 100 Munsell hues are identified simply by a number from 1 to 100, and on this scale hues that differ by 50 are nearly complementary. The most common convention, however, is to divide these 100 hues into 10 groups of 10 hues each, and identify each group by initials indicating the central member of the group, thus: red *R*, yellow red *YR*, yellow *Y*, green yellow *GY*, green *G*, blue green *BG*, blue *B*, purple blue *PB*, purple *P*, and red purple *RP*. The hues in each group are identified by the numbers 1 to 10. Thus, the most purplish of the red hues (1 on the scale of 100) is designated as 1*R*, the most yellowish as 10*R*, and the central hue as 5*R*, or often simply as *R;* see Figs. 9·30 and 9·31. The transition points (10*R*, 10*YR*, 10*Y*, and so on) between the groups of hues are also sometimes designated by means of the initials of the two adjacent hue groups, thus: *R–YR* = 10*R*, *YR–Y* = 10*YR*, *Y–GY* = 10*Y*, and so on; but this convention is little used. The Munsell notation is commonly written: Hue value/chroma, that is, the hue notation, such as 6*R*, then the value, such as 7, and finally the chroma, such as 4, the latter two being sep-

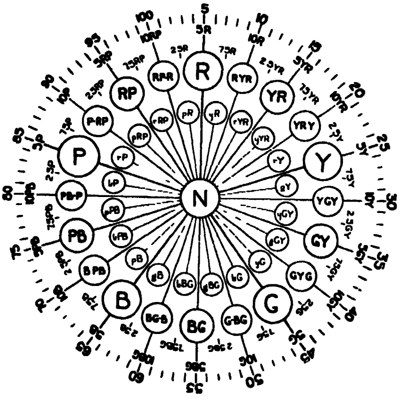


FIG. 9·30. Diagram of hue circle with Munsell hue notation.

arated by a shilling mark: $6R\ 7/4$. More precise designations are given in tenths of the arbitrary steps of the scales, thus: $6.2R\ 7.3/4.4$. The grays are indicated by the symbol N for neutral followed by the value notation, thus: $N\ 7/$ or $N\ 7.3/$; the chroma being zero for neutrals is not specifically noted.

Two representations of the Munsell system have been published, the original *Atlas* in 1915 (*70*), now chiefly a collector's item, and the *Munsell Book of Color* (*71*). This book consists of rectangles of hand-painted paper mounted on charts in a loose-leaf binding. The neutrals form a one-dimensional color scale extending from $N\ 1/$ to $N\ 9/$. Each chromatic sample, of which there are about 1000, takes its place on three color scales: a hue scale, a value scale, and a chroma scale; and the spacing of these scales is intended to be perceptually uniform. The pocket edition, adapted for determining Munsell notation of unknown colors by visual comparison, consists of forty constant-hue charts, so called because all the samples on each chart have the same Munsell hue. These samples are arranged in rows and columns, the rows being chroma scales at constant Munsell value, the columns being value scales at constant Munsell chroma. Comparison of an unknown color with these two families of scales gives by interpolation the Munsell value and Munsell chroma of the unknown. Interpolation between adjacent constant-hue charts gives the Munsell hue. Unknowns not too far outside the range of the Munsell charts may be evaluated with some reliability by extrapolation along the value and chroma scales. Table 9·13 gives *Munsell Book* notations of the four printing-ink specimens reproduced on Fig. 9·4. The greenish yellow specimen was evaluated by interpolation, the other three by extrapolation of varying degrees of uncertainty, the red-purple specimen being furthest outside the range of the Munsell charts and hence the least certainly evaluated by visual estimate.

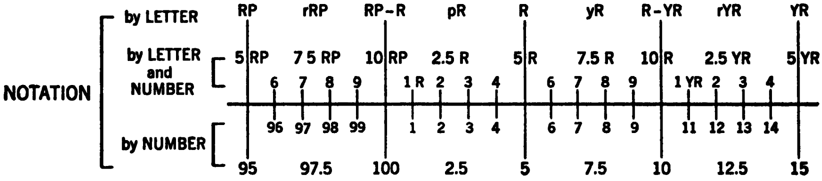

NOTATION
by LETTER
by LETTER and NUMBER
by NUMBER
RP rRP RP-R pR R yR R-YR rYR YR
5 RP 7.5 RP 10 RP 2.5 R 5 R 7.5 R 10 R 2.5 YR 5 YR
6 7 8 9 1 R 2 3 4 6 7 8 9 1 YR 2 3 4
96 97 98 99 1 2 3 4 6 7 8 9 11 12 13 14
95 97.5 100 2.5 5 7.5 10 12.5 15

FIG. 9·31. Alternate ways of expressing Munsell hue notation.

To facilitate the comparison of the unknown color with those of the paper rectangles on the Munsell charts, either the unknown color must be brought into juxtaposition with the rectangle and held in nearly the same plane, or, if the form of the unknown prevents such juxtaposition, two masks of thin cardboard having rectangular openings to fit the paper rectangles should be used. One mask should be placed over the unknown color; the other over one or another of the Munsell colors in succession to obtain the interpolated *Munsell Book* notation. Kelly (*50, 51*) made effective use of a form of mask with three rectangular openings particularly adapted to comparisons involving powdered chemicals and drugs viewed through a cover glass. It is advantageous to have the color of the mask fairly close to that of the unknown, particularly in Munsell value; that is, if the unknown color is dark, the mask should be of a dark color also. Use of a light mask for a dark color prevents the observer from making as precise a visual estimate as he can make with a mask more nearly a color match for the unknown.

TABLE 9·13 MUNSELL RENOTATIONS OF FOUR PRINTING-INK SPECIMENS FOUND FROM THEIR LUMINOUS REFLECTANCES AND CHROMATICITY COORDINATES; ALSO *Munsell Book* NOTATIONS, AND I.S.C.C.-N.B.S. COLOR DESIGNATIONS FOUND BY DIRECT VISUAL COMPARISON WITH THE *Munsell Book of Color*

Hue Designation of Specimen	Luminous Reflectance (Y/Y_0)	Chromaticity Coordinates		Munsell Renotation (Hue value/chroma)	*Munsell Book* Notation (Hue value/chroma)	I.S.C.C.-N.B.S. Color Designation
		x	y			
Red Purple	0.221	0.430	0.239	5.5*RP* $5.2_5/16._1$	6.0*RP* 4.8/16	Vivid red purple
Greenish Yellow	.704	.426	.476	8.3*Y* $8.6_0/10._6$	7.5*Y* 9.0/9.5	Brilliant greenish yellow
Greenish Blue	.242	.194	.248	5.6*B* $5.4_6/8._5$	3.0*B* 5.4/9	Strong greenish blue
Blue	.246	.190	.213	0.8*PB* $5.5_0/9._6$	10.0*B* 5.4/11	Strong blue

Because of the visual uniformity of the scales, the estimates of Munsell notation for unknown colors within the color range of the charts have a reliability corresponding to the use of a much larger collection of unequally spaced color standards. On this account the pocket edition of the *Munsell Book of Color* is widely used as a practical color standard for general purposes.

The standard or library edition shows the same colors as the pocket edition, but it shows them on constant-value charts in a polar coordinate system, and on constant-chroma charts in a rectangular coordinate system, as well as on constant-hue charts. This edition has full explanatory matter in the text and is adapted particularly for teaching color. It is too bulky for convenient practical use in determining the Munsell notation of an unknown color.

The samples of the 1929 *Munsell Book of Color* have been measured by means of the spectrophotometer twice independently with generally concordant results (*28*, *53*). In both these studies luminous reflectance, Y/Y_0, relative to magnesium oxide and chromaticity coordinates, x, y,

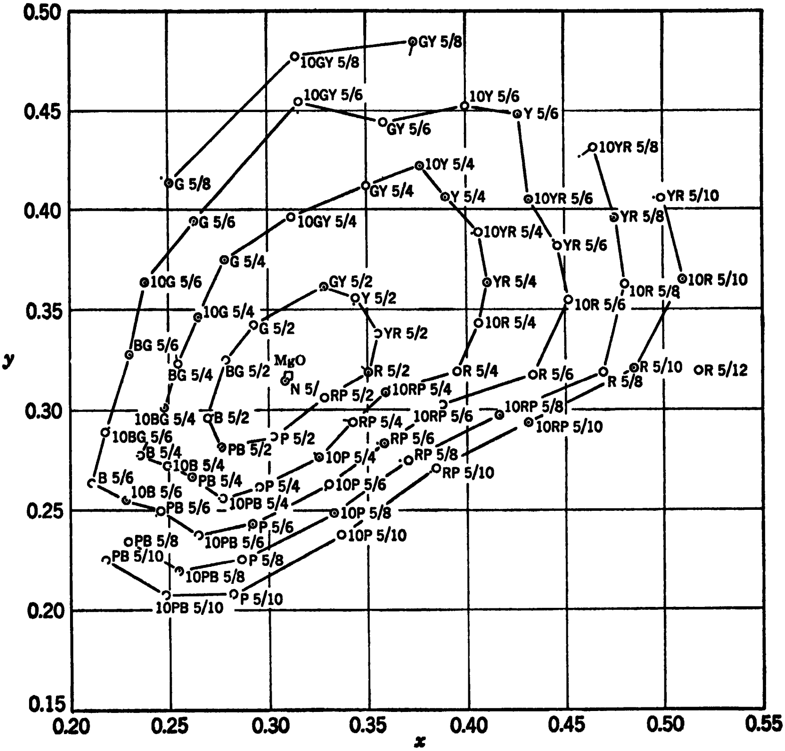


Fig. 9·32. Chromaticities of the standards of the *Munsell Book of Color*, value 5/. Results of measurements at the National Bureau of Standards are shown (*53*) by circles; those by Glenn and Killian (*28*) are shown as dots.

of the roughly 400 samples were computed for illuminant *C*. Glenn and Killian (*28*) have also published the dominant wavelength and purity for each of the colors; and Kelly, Gibson, and Nickerson (*53*) have published specifications (Y, x, y) for three additional illuminants (illuminant *A*, Macbeth daylight, and limit blue sky). Furthermore, they have published a series of (x, y) chromaticity diagrams showing the position of the Munsell colors for each of the Munsell values from 2/ through 8/. Figure 9·32 is their diagram for Munsell value 5/. From these diagrams it is possible to find the chromaticity coordinates, x, y, corresponding to any *Munsell Book* notation; and the reverse transformation is also possible. The samples of the 1942 supplement to the *Munsell Book of Color* together with many special Munsell standards have been measured spectrophotometrically by Granville, Nickerson, and Foss (*34*). These Munsell standards, together with those of the 1929 *Munsell Book of Color*, number over 1000 and comprise the largest systematic set of color standards of known luminous reflectance and chromaticity coordinates ever made. These standards are commercially available separately in disc form and on large sheets, and they make practical the general colorimetry of opaque specimens not only by disc mixture (see Figs. 9·8 and 9·9), but also by difference from a standard (see Division C).

From the charts themselves, luminous reflectance and chromaticity coordinates of an unknown color may also be found quickly and with an accuracy sufficient for many purposes by obtaining first the *Munsell Book* notation of the unknown and then transforming it by reference to (x, y) interpolation charts (like Fig. 9·32) based on the complete set of standards.

The Munsell color standards may also be used, though less conveniently, in the colorimetry of light-transmitting (*50, 51*) elements (gelatin films, crystal and glass plates, solutions, and so on); and, conversely, such elements may be given *Munsell Book* notations from their I.C.I. specifications by means of interpolation charts like Fig. 9·32. Table 9·16, to be discussed presently in another connection, shows *Munsell Book* notations so derived from the luminous transmittances, T, and chromaticity coordinates, x, y, of the glass standards of the A.S.T.M. Union colorimeter (see Table 9·10). The last four book notations given are relatively uncertain because the colors to be specified are far outside the range of the Munsell standards.

The spacing of the Munsell colors has been examined in detail by a subcommittee of the Colorimetry Committee of the Optical Society of America (*75*). This committee work confirmed the many local irregularities in spacing revealed by the spectrophotometric studies (see Fig. 9·32) and established the need for some more general but minor adjustments to make the colors of the Munsell charts correlate more nearly perfectly under ordinary observing conditions (adaptation to daylight, gray surrounding field, and so on) with the surface-color solid. The subcommittee found it possible from this study to recommend specifications (Y, x, y) on the standard coordinate system defining an ideal Munsell system (*76*). It has also given to every Munsell standard a revised notation, called the *Munsell renotation*, indicating exactly in what way and how much each color chip deviates from the ideal. Furthermore, the recommended definition of the ideal Munsell system has been extended beyond the color ranges covered by the present Munsell charts so as to include all colors theoretically producible from nonfluorescent materials (*60*) under illuminant *C*. The connection between luminous reflectance, Y, and Munsell renotation value is given in Table 9·14. Note that N 0/ corresponds to $Y = 0$, N 9·91/ corresponds to

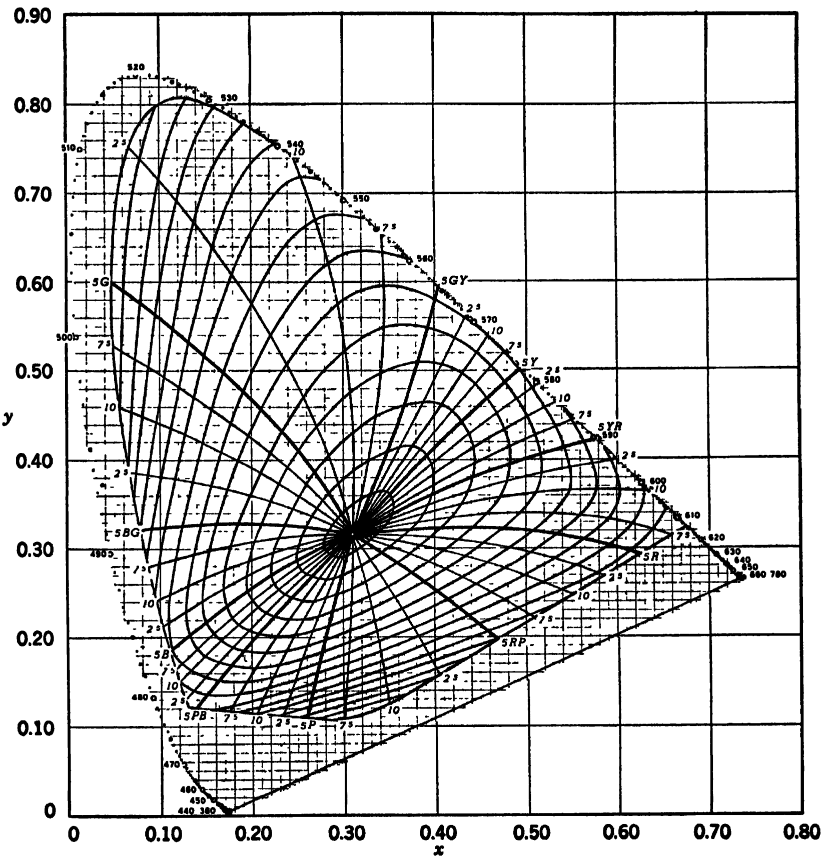


FIG. 9·33. Chromaticities of ideal Munsell colors, value 5/, shown on the (x, y) diagram. This chart serves to define Munsell renotation hue and chroma for colors having $Y/Y_0 = 0.198$. (Prepared by Color Measurement Laboratory, War Food Administration, U.S.D.A.)

magnesium oxide ($Y = 1.00$), and N 5.0/ corresponds to $Y = 0.198$ (quite different from 0.50, the half-way point). Figure 9·33 shows one of the (x, y)-chromaticity charts (that for Munsell renotation value equal to 5.0) defining the ideal system. From these charts combined with the data given in Table 9·14 it is possible to find the ideal Munsell notation for any color specified in terms of luminous reflectance, Y, and

TABLE 9·14 I.C.I. (Y) EQUIVALENTS (IN PER CENT RELATIVE TO MgO) OF THE RECOMMENDED MUNSELL VALUE SCALE (V) FROM 0/ TO 10/

V	Y_V	V	Y_V	V	Y_V	V	Y_V	V	Y_V
10.00	102.56	. .	. . . . .	. .	. . . .	. .	. . . .	. . .	. . . .
9.99	102.30	9.49	89.77	8.99	78.45	8.49	68.20	7.99	58.92
8	102.04	8	89.53	8	78.23	8	68.01	8	58.74
7	101.78	7	89.30	7	78.02	7	67.81	7	58.57
6	101.52	6	89.06	6	77.80	6	67.62	6	58.39
5	101.25	5	88.82	5	77.59	5	67.43	5	58.22
9.94	100.99	9.44	88.59	8.94	77.38	8.44	67.23	7.94	58.04
3	100.73	3	88.35	3	77.16	3	67.04	3	57.87
2	100.47	2	88.12	2	76.95	2	66.85	2	57.69
1	100.21	1	87.88	1	76.74	1	66.66	1	57.52
0	99.95	0	87.65	0	76.53	0	66.46	0	57.35
9.89	99.69	9.39	87.41	8.89	76.32	8.39	66.27	7.89	57.17
8	99.44	8	87.18	8	76.11	8	66.08	8	57.00
7	99.18	7	86.95	7	75.90	7	65.89	7	56.83
6	98.92	6	86.72	6	75.69	6	65.70	6	56.66
5	98.66	5	86.48	5	75.48	5	65.51	5	56.48
9.84	98.41	9.34	86.25	8.84	75.27	8.34	65.32	7.84	56.31
3	98.15	3	86.02	3	75.06	3	65.13	3	56.14
2	97.90	2	85.79	2	74.85	2	64.94	2	55.97
1	97.64	1	85.56	1	74.64	1	64.76	1	55.80
0	97.39	0	85.33	0	74.44	0	64.57	0	55.63
9.79	97.14	9.29	85.10	8.79	74.23	8.29	64.38	7.79	55.46
8	96.88	8	84.88	8	74.02	8	64.19	8	55.29
7	96.63	7	84.65	7	73.82	7	64.01	7	55.12
6	96.38	6	84.42	6	73.61	6	63.82	6	54.95
5	96.13	5	84.19	5	73.40	5	63.63	5	54.78
9.74	95.88	9.24	83.97	8.74	73.20	8.24	63.45	7.74	54.62
3	95.63	3	83.74	3	72.99	3	63.26	3	54.45
2	95.38	2	83.52	2	72.79	2	63.08	2	54.28
1	95.13	1	83.29	1	72.59	1	62.89	1	54.11
0	94.88	0	83.07	0	72.38	0	62.71	0	53.94
9.69	94.63	9.19	82.84	8.69	72.18	8.19	62.52	7.69	53.78
8	94.38	8	82.62	8	71.98	8	62.34	8	53.61
7	94.14	7	82.39	7	71.78	7	62.16	7	53.45
6	93.89	6	82.17	6	71.57	6	61.98	6	53.28
5	93.64	5	81.95	5	71.37	5	61.79	5	53.12
9.64	93.40	9.14	81.73	8.64	71.17	8.14	61.61	7.64	52.95
3	93.15	3	81.50	3	70.97	3	61.43	3	52.79
2	92.91	2	81.28	2	70.77	2	61.25	2	52.62
1	92.66	1	81.06	1	70.57	1	61.07	1	52.46
0	92.42	0	80.84	0	70.37	0	60.88	0	52.30
9.59	92.18	9.09	80.62	8.59	70.17	8.09	60.70	7.59	52.13
8	91.93	8	80.40	8	69.97	8	60.52	8	51.97
7	91.69	7	80.18	7	69.78	7	60.35	7	51.81
6	91.45	6	79.97	6	69.58	6	60.17	6	51.64
5	91.21	5	79.75	5	69.38	5	59.99	5	51.48
9.54	90.97	9.04	79.53	8.54	69.18	8.04	59.81	7.54	51.32
3	90.73	3	79.31	3	68.99	3	59.63	3	51.16
2	90.49	2	79.10	2	68.79	2	59.45	2	51.00
1	90.25	1	78.88	1	68.59	1	59.28	1	50.84
0	90.01	0	78.66	0	68.40	0	59.10	0	50.68

TABLE 9·14 I.C.I. (Y) EQUIVALENTS (IN PER CENT RELATIVE TO MgO) OF THE RECOMMENDED MUNSELL VALUE SCALE (V) FROM 0/ TO 10/ (*Continued*)

V	Y_V	V	Y_V	V	Y_V	V	Y_V	V	Y_V
7.49	50.52	6.99	42.92	6.49	36.07	5.99	29.94	5.49	24.48
8	50.36	8	42.77	8	35.94	8	29.82	8	24.38
7	50.20	7	42.63	7	35.81	7	29.71	7	24.28
6	50.04	6	42.49	6	35.68	6	29.59	6	24.17
5	49.88	5	42.34	5	35.56	5	29.48	5	24.07
7.44	49.72	6.94	42.20	6.44	35.43	5.94	29.36	5.44	23.97
3	49.56	3	42.06	3	35.30	3	29.25	3	23.87
2	49.41	2	41.92	2	35.17	2	29.13	2	23.77
1	49.25	1	41.77	1	35.04	1	29.02	1	23.67
0	49.09	0	41.63	0	34.92	0	28.90	0	23.57
7.39	48.93	6.89	41.49	6.39	34.79	5.89	28.79	5.39	23.47
8	48.78	8	41.35	8	34.66	8	28.68	8	23.37
7	48.62	7	41.21	7	34.54	7	28.57	7	23.27
6	48.47	6	41.07	6	34.41	6	28.45	6	23.17
5	48.31	5	40.93	5	34.28	5	28.34	5	23.07
7.34	48.16	6.84	40.79	6.34	34.16	5.84	28.23	5.34	22.97
3	48.00	3	40.65	3	34.03	3	28.12	3	22.87
2	47.85	2	40.51	2	33.91	2	28.01	2	22.78
1	47.69	1	40.37	1	33.78	1	27.90	1	22.68
0	47.54	0	40.23	0	33.66	0	27.78	0	22.58
7.29	47.38	6.79	40.09	6.29	33.54	5.79	27.67	5.29	22.48
8	47.23	8	39.95	8	33.41	8	27.56	8	22.38
7	47.08	7	39.82	7	33.29	7	27.45	7	22.29
6	46.92	6	39.68	6	33.16	6	27.34	6	22.19
5	46.77	5	39.54	5	33.04	5	27.23	5	22.09
7.24	46.62	6.74	39.40	6.24	32.92	5.74	27.12	5.24	22.00
3	46.47	3	39.27	3	32.80	3	27.02	3	21.90
2	46.32	2	39.13	2	32.67	2	26.91	2	21.81
1	46.17	1	39.00	1	32.55	1	26.80	1	21.71
0	46.02	0	38.86	0	32.43	0	26.69	0	21.62
7.19	45.87	6.69	38.72	6.19	32.31	5.69	26.58	5.19	21.52
8	45.72	8	38.59	8	32.19	8	26.48	8	21.43
7	45.57	7	38.45	7	32.07	7	26.37	7	21.33
6	45.42	6	38.32	6	31.95	6	26.26	6	21.24
5	45.27	5	38.18	5	31.83	5	26.15	5	21.14
7.14	45.12	6.64	38.05	6.14	31.71	5.64	26.05	5.14	21.05
3	44.97	3	37.92	3	31.59	3	25.94	3	20.96
2	44.82	2	37.78	2	31.47	2	25.84	2	20.86
1	44.67	1	37.65	1	31.35	1	25.73	1	20.77
0	44.52	0	37.52	0	31.23	0	25.62	0	20.68
7.09	44.38	6.59	37.38	6.09	31.11	5.59	25.52	5.09	20.59
8	44.23	8	37.25	8	30.99	8	25.41	8	20.49
7	44.08	7	37.12	7	30.87	7	25.31	7	20.40
6	43.94	6	36.99	6	30.75	6	25.20	6	20.31
5	43.79	5	36.86	5	30.64	5	25.10	5	20.22
7.04	43.64	6.54	36.72	6.04	30.52	5.54	25.00	5.04	20.13
3	43.50	3	36.59	3	30.40	3	24.89	3	20.04
2	43.35	2	36.46	2	30.28	2	24.79	2	19.95
1	43.21	1	36.33	1	30.17	1	24.69	1	19.86
0	43.06	0	36.20	0	30.05	0	24.58	0	19.77

TABLE 9·14 I.C.I. (*Y*) EQUIVALENTS (IN PER CENT RELATIVE TO MgO) OF THE RECOMMENDED MUNSELL VALUE SCALE (*V*) FROM 0/ TO 10/ (*Continued*)

V	Y_V	V	Y_V	V	Y_V	V	Y_V	V	Y_V
4.99	19.68	4.49	15.49	3.99	11.935	3.49	8.949	2.99	6.511
8	19.59	8	15.42	8	11.870	8	8.895	8	6.468
7	19.50	7	15.34	7	11.805	7	8.841	7	6.425
6	19.41	6	15.26	6	11.740	6	8.787	6	6.382
5	19.32	5	15.18	5	11.675	5	8.734	5	6.339
4.94	19.23	4.44	15.11	3.94	11.611	3.44	8.681	2.94	6.296
3	19.14	3	15.03	3	11.547	3	8.628	3	6.254
2	19.06	2	14.96	2	11.483	2	8.575	2	6.212
1	18.97	1	14.88	1	11.419	1	8.523	1	6.170
0	18.88	0	14.81	0	11.356	0	8.471	0	6.128
4.89	18.79	4.39	14.73	3.89	11.292	3.39	8.419	2.89	6.086
8	18.70	8	14.66	8	11.229	8	8.367	8	6.045
7	18.62	7	14.58	7	11.167	7	8.316	7	6.003
6	18.53	6	14.51	6	11.104	6	8.264	6	5.962
5	18.44	5	14.43	5	11.042	5	8.213	5	5.921
4.84	18.36	4.34	14.36	3.84	10.980	3.34	8.162	2.84	5.881
3	18.27	3	14.28	3	10.918	3	8.111	3	5.841
2	18.19	2	14.21	2	10.856	2	8.060	2	5.800
1	18.10	1	14.14	1	10.795	1	8.010	1	5.760
0	18.02	0	14.07	0	10.734	0	7.960	0	5.720
4.79	17.93	4.29	13.99	3.79	10.673	3.29	7.910	2.79	5.680
8	17.85	8	13.92	8	10.612	8	7.860	8	5.641
7	17.76	7	13.85	7	10.551	7	7.811	7	5.602
6	17.68	6	13.78	6	10.491	6	7.762	6	5.563
5	17.60	5	13.70	5	10.431	5	7.713	5	5.524
4.74	17.51	4.24	13.63	3.74	10.371	3.24	7.664	2.74	5.485
3	17.43	3	13.56	3	10.311	3	7.615	3	5.447
2	17.34	2	13.49	2	10.252	2	7.567	2	5.408
1	17.26	1	13.42	1	10.193	1	7.519	1	5.370
0	17.18	0	13.35	0	10.134	0	7.471	0	5.332
4.69	17.10	4.19	13.28	3.69	10.075	3.19	7.423	2.69	5.295
8	17.02	8	13.21	8	10.017	8	7.375	8	5.257
7	16.93	7	13.14	7	9.959	7	7.328	7	5.220
6	16.85	6	13.07	6	9.901	6	7.281	6	5.183
5	16.77	5	13.00	5	9.843	5	7.234	5	5.146
4.64	16.69	4.14	12.93	3.64	9.785	3.14	7.187	2.64	5.109
3	16.61	3	12.86	3	9.728	3	7.140	3	5.072
2	16.53	2	12.80	2	9.671	2	7.094	2	5.036
1	16.45	1	12.73	1	9.614	1	7.048	1	5.000
0	16.37	0	12.66	0	9.557	0	7.002	0	4.964
4.59	16.29	4.09	12.59	3.59	9.501	3.09	6.956	2.59	4.928
8	16.21	8	12.52	8	9.445	8	6.911	8	4.892
7	16.13	7	12.46	7	9.389	7	6.866	7	4.857
6	16.05	6	12.39	6	9.333	6	6.821	6	4.822
5	15.97	5	12.32	5	9.277	5	6.776	5	4.787
4.54	15.89	4.04	12.26	3.54	9.222	3.04	6.731	2.54	4.752
3	15.81	3	12.19	3	9.167	3	6.687	3	4.717
2	15.74	2	12.12	2	9.112	2	6.643	2	4.682
1	15.66	1	12.06	1	9.058	1	6.599	1	4.648
0	15.57	0	12.00	0	9.003	0	6.555	0	4.614

TABLE 9·14 I.C.I. (Y) EQUIVALENTS (IN PER CENT RELATIVE TO MgO) OF THE RECOMMENDED MUNSELL VALUE SCALE (V) FROM 0/ TO 10/ (*Continued*)

V	Y_V	V	Y_V	V	Y_V	V	Y_V	V	Y_V
2.49	4.580	1.99	3.100	1.49	2.002	0.99	1.196	0.49	0.570
8	4.546	8	3.075	8	1.983	8	1.182	8	.559
7	4.512	7	3.050	7	1.965	7	1.168	7	.547
6	4.479	6	3.025	6	1.947	6	1.154	6	.535
5	4.446	5	3.000	5	1.929	5	1.141	5	.524
2.44	4.413	1.94	2.975	1.44	1.910	0.94	1.128	0.44	.513
3	4.380	3	2.950	3	1.892	3	1.114	3	.501
2	4.347	2	2.925	2	1.874	2	1.101	2	.489
1	4.314	1	2.901	1	1.856	1	1.087	1	.478
0	4.282	0	2.877	0	1.838	0	1.074	0	.467
2.39	4.250	1.89	2.853	1.39	1.821	0.89	1.060	0.39	.455
8	4.218	8	2.829	8	1.803	8	1.047	8	.444
7	4.186	7	2.805	7	1.786	7	1.034	7	.432
6	4.154	6	2.781	6	1.769	6	1.021	6	.421
5	4.123	5	2.758	5	1.752	5	1.008	5	.409
2.34	4.092	1.84	2.735	1.34	1.735	0.84	0.995	0.34	.398
3	4.060	3	2.712	3	1.718	3	.982	3	.386
2	4.029	2	2.688	2	1.701	2	.969	2	.375
1	3.998	1	2.665	1	1.684	1	.956	1	.363
0	3.968	0	2.642	0	1.667	0	.943	0	.352
2.29	3.938	1.79	2.620	1.29	1.650	0.79	.931	0.29	.341
8	3.907	8	2.598	8	1.634	8	.918	8	.329
7	3.877	7	2.575	7	1.618	7	.906	7	.318
6	3.847	6	2.553	6	1.601	6	.893	6	.306
5	3.817	5	2.531	5	1.585	5	.881	5	.295
2.24	3.787	1.74	2.509	1.24	1.569	0.74	.868	0.24	.283
3	3.758	3	2.487	3	1.553	3	.856	3	.272
2	3.729	2	2.465	2	1.537	2	.844	2	.260
1	3.700	1	2.443	1	1.521	1	.832	1	.248
0	3.671	0	2.422	0	1.506	0	.819	0	.237
2.19	3.642	1.69	2.401	1.19	1.490	0.69	.807	0.19	.225
8	3.613	8	2.380	8	1.475	8	.795	8	.214
7	3.585	7	2.359	7	1.459	7	.783	7	.202
6	3.557	6	2.338	6	1.444	6	.771	6	.191
5	3.529	5	2.317	5	1.429	5	.759	5	.179
2.14	3.501	1.64	2.296	1.14	1.413	0.64	.747	0.14	.167
3	3.473	3	2.276	3	1.398	3	.735	3	.155
2	3.445	2	2.256	2	1.383	2	.723	2	.143
1	3.418	1	2.236	1	1.368	1	.711	1	.131
0	3.391	0	2.216	0	1.354	0	.699	0	.120
2.09	3.364	1.59	2.196	1.09	1.339	0.59	.687	0.09	.108
8	3.337	8	2.176	8	1.324	8	.675	8	.096
7	3.310	7	2.156	7	1.310	7	.663	7	.084
6	3.283	6	2.136	6	1.295	6	.651	6	.073
5	3.256	5	2.116	5	1.281	5	.640	5	.061
2.04	3.230	1.54	2.097	1.04	1.267	0.54	.628	0.04	.049
3	3.204	3	2.078	3	1.253	3	.617	3	.036
2	3.178	2	2.059	2	1.238	2	.605	2	.024
1	3.152	1	2.040	1	1.224	1	.593	1	.012
0	3.126	0	2.021	0	1.210	0	.581	0	.000

chromaticity coordinates, x, y. Table 9·13 shows these Munsell renotations for the four printing-ink specimens of Fig. 9·4. Note that they agree in a general way, though not perfectly, with the notations found by direct visual comparison with the *Munsell Book of Color*. Some of the discrepancies are ascribable to local irregularities of the color spacing of the Munsell charts, but most of them may be laid to the uncertainty of the visual estimates, all but that for the greenish yellow specimen requiring extrapolation over a considerable color range.

Munsell renotations, such as these, have a unique usefulness as color specifications. Because of their definition in terms of the standard I.C.I. coordinate system they are capable of nearly the precision of the Y, x, y form of specification, and like that form they may be extended to apply to all object colors, both opaque and transparent objects. For opaque surfaces Y/Y_0 is luminous reflectance relative to magnesium oxide; for transparent objects Y/Y_0 is luminous transmittance relative to an equivalent thickness of air; for solutions Y/Y_0 is luminous transmittance relative to the same thickness of distilled water or solvent. For any of these objects Munsell renotation value, V, may be found from Y/Y_0 in accord with Table 9·14. Because of their close correlation with the color solid, Munsell renotations are capable of being quickly understood. Thus the renotation $8.3Y$ $8.6_0/10._6$ for the greenish yellow printing-ink specimen indicates from the letter Y that the specimen is a yellow, from the value 8.6_0 that it is a relatively light color, being close to the top of the value scale, 0 to 10, and from the chroma $10._6$, that it is a strong or nongrayish color, being more than 10 Munsell chroma steps away from the gray of the same Munsell value. Munsell renotation hue and chroma serve more adequately for object colors the purposes formerly served by dominant wavelength and purity. Munsell renotation hue correlates significantly better under ordinary conditions of daylight observation with the hue of the perceived color than does dominant wavelength, and Munsell renotation chroma is by far superior to purity in its correlation to saturation. This correlation with the color-perception solid does not, however, necessarily hold under all observing conditions. Ordinarily this printing-ink specimen will be perceived to have a light, strong greenish yellow color, but it is not so perceived under all conditions. If this specimen be viewed next to a brilliant yellow-green area such as is provided by a fluorescent fabric, it will be perceived to take on a darker color of yellowish orange hue and moderate saturation. Thus the lightness, hue, and saturation of the color perception depend upon the surroundings and upon the adaptive state of the eye; and lightness, hue, and saturation are taken correctly to be psychological terms. But the Munsell renotation refers only to the light that is reflected from the specimen and stays constant regardless of these changes in observing conditions. It is therefore a psychophysical characterization of the specimen according to the light reflected from it (see Division *F*), just as are luminous reflectance, Y, and chromaticity coordinates, x, y, from which it can be derived, and dominant wavelength and purity which it is coming to supplant.

Another advantage of expressing spectrophotometric results in the form of the Munsell renotation is that the amount and kind of the color difference between two specimens can be found immediately from the two renotations in an easily understandable form. Thus, from the hue difference between the two blue printing-ink specimens (5.6*B* compared to 0.8*PB*), the former is seen to be more greenish (less purplish) by about five Munsell hue steps. From the value difference (5.4_6 compared to 5.5_0) the two are seen to be of the same value to the nearest one-tenth Munsell value step; and from the chroma difference (8.5 compared to 9.6) the greenish blue is seen to be more grayish by one Munsell chroma step. Such differences as these in terms of Munsell hue, value, and chroma may be combined into a single index, I, of color difference (*79*, *80*, *82*):

$$I = \frac{C}{5}(2\Delta \mathrm{H}) + 6\Delta V + 3\Delta C \tag{9·7}$$

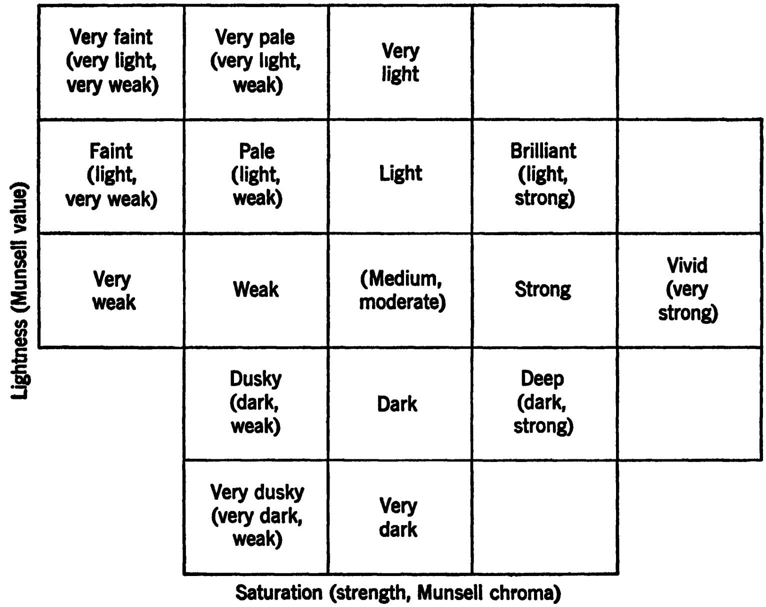
where C is Munsell chroma, and ΔH, ΔV, ΔC, are the differences between the two colors in Munsell hue, value, and chroma, respectively. The difference between the colors of the two blue printing-ink specimens would be found by this formula as:

$$\begin{aligned} I &= \left(\frac{9.0}{5}\right)(2 \times 5.2) + 6 \times 0.04 + 3 \times 1.1 \\ &= 18.7 + 0.2 + 3.3 = 22.2 \text{ units} \end{aligned}$$

These units are of such size that color differences of less than one unit would ordinarily not be of commercial importance; that is, pairs of colors exhibiting such differences would be considered to be commercial matches. Note that these two blue printing-ink specimens are far from being a commercial match; also note that the hue difference is by far more important than the chroma difference, and that the value equivalence is well within commercial toleration.

9·33 I.S.C.C.-N.B.S. method of designating colors. A method devised at the request of the American Pharmaceutical Association and the United States Pharmacopoeial Convention for designating the colors of drugs and chemicals is coming into use for general purposes. The general plan of the method was worked out by the Inter-Society Color

Council, and the details were developed at the National Bureau of Standards; the method is therefore referred to as the I.S.C.C.-N.B.S. method of designating colors (*50*, *51*). This method provides a designation for every color perceived as belonging to an object (either an opaque surface, or a light-transmitting layer), and it has been extended to the colors of self-luminous areas by Kelly (*52*); see Fig. 9·3. The number of color designations was purposely made small (slightly over 300) for the sake of simplicity. Since about ten million surface colors can be distinguished by the normal human observer with optimum observing conditions, the I.S.C.C.-N.B.S. method falls far short of supplying a different designation for each distinguishable color, or even for all colors (numbering perhaps half a million) considered to be commercially different.


Lightness (Munsell value)
Saturation (strength, Munsell chroma)

Very faint (very light, very weak)	Very pale (very light, weak)	Very light		
Faint (light, very weak)	Pale (light, weak)	Light	Brilliant (light, strong)	
Very weak	Weak	(Medium, moderate)	Strong	Vivid (very strong)
	Dusky (dark, weak)	Dark	Deep (dark, strong)	
	Very dusky (very dark, weak)	Very dark		

FIG. 9·34. I.S.C.C.-N.B.S. system of modifiers used in forming color designations.

The plan of the method is to divide the surface-color solid (see Fig. 9·29) arbitrarily into about 300 compartments, and assign a designation to each in as good conformity as possible to color nomenclature currently used in art, science, and industry. The compartments embracing the black-white axis are given the following designations: black, dark gray, medium gray, light gray, and white. The compartments adjacent to these are given similar designations formed by adding an adjective indicating the hue, such as yellowish white, dark purplish gray, or greenish black. All other compartments take designations consisting of a hue name (red, orange, yellow, green, blue, purple, pink, brown, olive) preceded by modifiers (light, dark, weak, strong) indicating the lightness and saturation of the perceived color. Figure 9·34 shows the complete list of modifiers used in the I.S.C.C.-N.B.S. system; note that *vivid* is a substitute for *very strong; pale*, a substitute for *light, weak; deep*, for *dark, strong*, and so on.

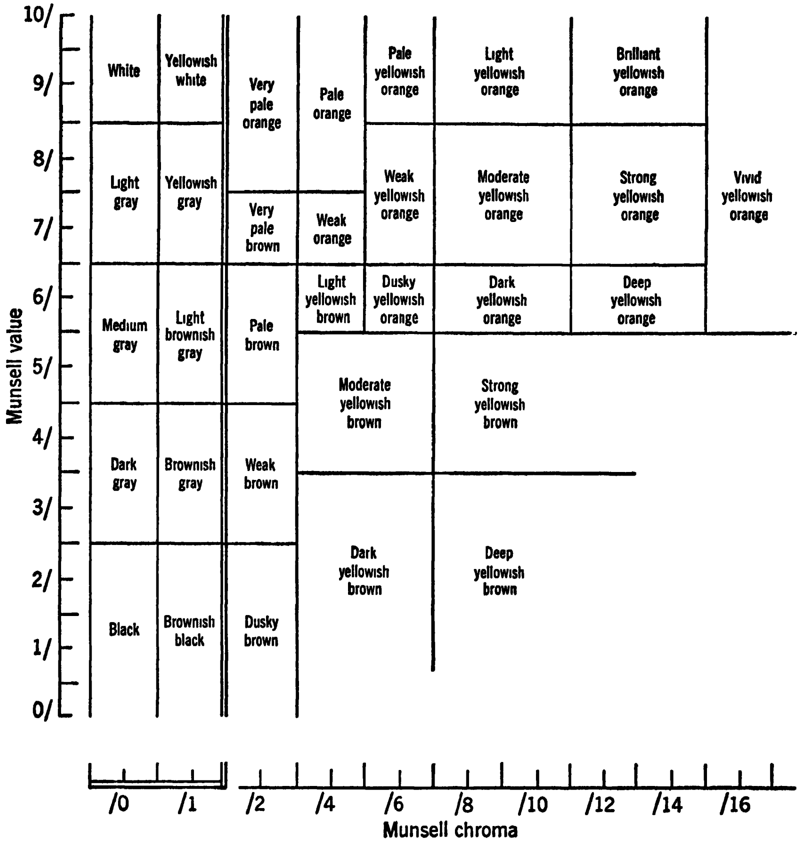


FIG. 9·35. I.S.C.C.-N.B.S. color designations for Munsell hue 9*YR*.

The boundaries between the groups of colors known by these designations have been adjusted to accord as closely as possible with common usage and have been expressed in terms of *Munsell Book* notation. Figure 9·35 shows one of the charts indicating these boundaries, and

Fig. 9·36 indicates the shape of the compartments selected for colors of purple hue.

The I.S.C.C.-N.B.S. designations are not to be considered a substitute for numerical designation of color resulting from application of a suitable colorimetric method, but they do supply a certain precision to ordinary color designations that has previously been lacking. Table 9·13 gives the I.S.C.C-N.B.S. designations of the four printing-ink

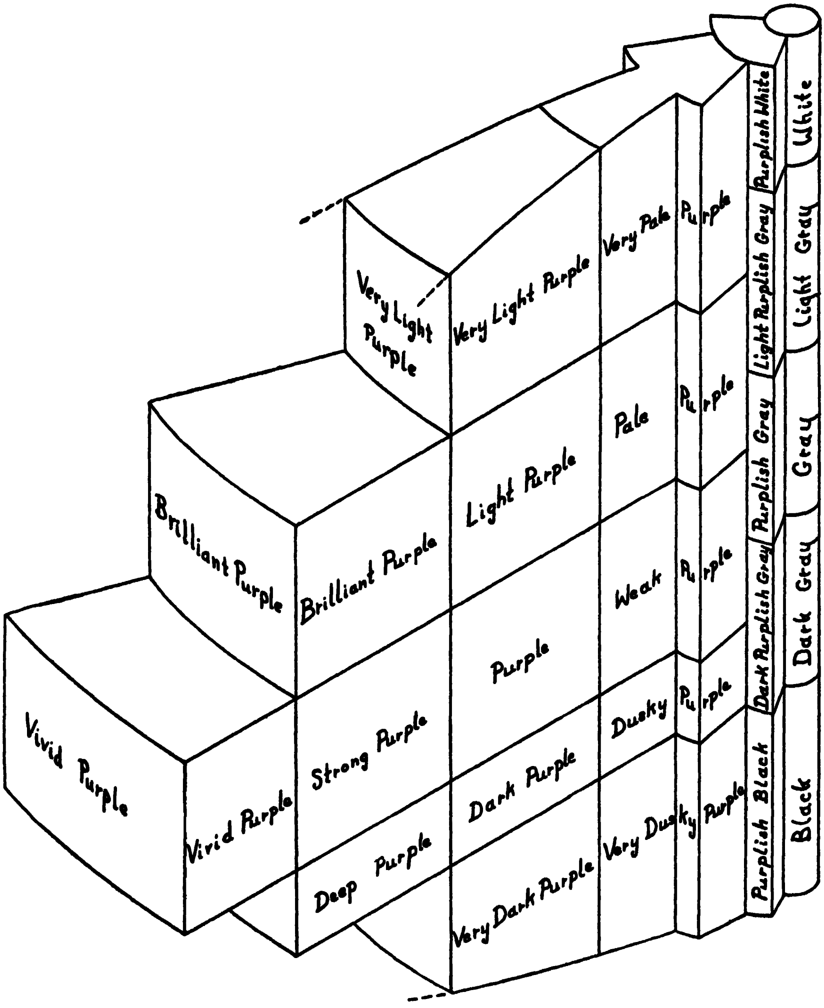


FIG. 9·36. Sketch indicating the shape of the compartments selected in the color solid for I.S.C.C.-N.B.S. designations of colors of purple hue.

specimens shown on Fig. 9·4, and the I.S.C.C.-N.B.S. hue designations have been used throughout the text of this chapter. These color designations are used in the current edition (N.F. VII) of the National Formulary, and they are being inserted into the United States Pharmacopoeia and into a textbook of qualitative analysis (*88*). They have been used for describing the colors of building stone (*54*) and soils (*96*), and for a considerable variety of research purposes such as the description of mica colors after heat treatment (*39*). Nickerson and Newhall (*81*) have found the *Munsell Book* notations for the central color of each compartment assigned an I.S.C.C.-N.B.S. color designation and have recommended a system of abbreviations; see Table 9·15.

TABLE 9·15 ABBREVIATIONS FOR I.S.C.C.-N.B.S. COLOR DESIGNATIONS (*81*)

B = blue
b = bluish
Bk = black
Br = brown
br = brownish
d = dusky
dk = dark
G = green
g = greenish
Gr = gray

lt = light
med = medium
mod = moderate
O = orange
0 = orange (adj.)
Ol = olive
ol = olive (adj.)
P = purple
p = purplish
Pk = pink

pk = pinkish
R = red
r = reddish
str = strong
v = very
viv = vivid
Wh = white
wk = weak
Y = yellow
y = yellowish

The I.S.C.C.-N.B.S. designations are generally unsuited for use in sales promotion in which it is important to avoid any suggestion of weakness or adulteration. To illustrate this point the *Munsell Book* notations corresponding to the I.C.I. specifications for the glass standards of the A.S.T.M. Union colorimeter (see Table 9·10) have been found by interpolation and extrapolation on charts like Fig. 9·31, and the corresponding I.S.C.C.-N.B.S. color designations have been read (*50*) and listed in Table 9·16. It will be noted that these designations give a satisfactory description of the colors of petroleum oil, but the term *brown* suggests mud or impurities and would probably never be applied by an oil dealer to his own product; compare these designations with the corresponding names used by the National Petroleum Association (see first column of table). Furthermore, a light-colored oil suggests extreme purity; so names emphasizing the paleness of the color tend to become current in trade; note the first eight names in the N.P.A. list. The I.S.C.C.-N.B.S. color designations are intended to be as descriptive as possible; they are not intended to supplant color names adopted for sales promotion.

The I.S.C.C.-N.B.S. method has been approved for color description of drugs and chemicals by the delegates of nine national societies, and it has been recommended for general use by the American Standards Association in the *American War Standard for the Description and Specification of Color* Z44-1942.

TABLE 9·16 GLASS STANDARDS OF THE A.S.T.M. UNION COLORIMETER

	I.C.I. Specification				
National Petroleum Association Names	Luminous Transmittance, (*T*)	Chromaticity Coordinates		*Munsell Book Notation* (Hue value/chroma)	*I.S.C.C.-N.B.S. Color Designation*
		x	*y*		
Lily white	0.751	0.349	0.382	10*Y* 9.6/3.8	Pale greenish yellow
Cream white	.654	.400	.446	8*Y* 8.8/8.0	Light greenish yellow
Extra pale	.443	.472	.476	3*Y* 7.2/12	Strong yellow
Extra lemon pale	.365	.498	.457	10*YR* 6.0/12	Deep yellowish orange
Lemon pale	.287	.525	.440	7*YR* 5.7/13.5	Strong orange
Extra orange pale	.211	.556	.423	4*YR* 5.0/14	Deep orange
Orange pale	.096	.591	.400	2*YR* 3.2/12	Strong brown
Pale	.065	.620	.376	1*YR* 2.6/12	Strong reddish brown
Light red	.036	.653	.347	1*YR* 2.2/9.5	Deep reddish brown
Dark red	.017	.676	.323	1*YR* 1.3/8.5	Dark reddish brown
Claret red	.0066	.684	.316	1*YR* 0.6/4.5	Dusky reddish brown
....	.0020	.714	.286	1*YR* 0.2/1.4	Reddish black

E·SUMMARY

9·34 General recommendations. In 1942 there was approved and published by the American Standards Association an *American War Standard for the Specification and Description of Color* under the designation ASA Z44-1942 (*5*). This standard is of importance because it points the way toward agreement on basic procedures in colorimetry. It also serves well as a summary of this chapter. Hence the text, with foreword and notes deleted, and literature references brought up to date, is reproduced here verbatim.

Purpose. To recognize and recommend a basic method for the specification of color, and to facilitate its popular interpretation.

Provisions. (1) The spectrophotometer shall be recognized as the basic instrument in the fundamental standardization of color.

(2) Color specifications computed from spectrophotometric data shall be found by means of the standard observer and coordinate system adopted in 1931 by the International Commission on Illumination (*36*, *45*, *93*).

In the absence of a special reason for adopting some other illuminant in reducing spectrophotometric data, standard I.C.I. illuminant *C*, representative of average daylight, shall be used (*36*, *45*, *93*).

The basic specifications of color shall consist of the tristimulus value, *Y*, and the trichromatic coefficients, *x* and *y*, of the I.C.I. coordinate system, or they shall consist of the tristimulus value, *Y*, and the dominant wavelength and purity (*36*, *45*).

(3) For the popular identification of color, material standards may be used. The only system of material standards that has been calibrated in terms of the basic specification is represented by the 1929 edition of the *Munsell Book of Color* (*71*, *28*, *53*, *34*). The use of this book is recommended wherever applicable to the specification of the color of surfaces. Approximate identifications of Munsell hue, value, and chroma may be secured by direct visual comparison with the samples in the 1929 *Munsell Book of Color.* When the most accurate visual comparisons are needed, the mask method (*50*, *51*, *80*) is recommended. Wherever more exact Munsell notations are desired, they shall be found from the basic specification, *Y*, *x*, and *y*, by interpolation among the smoothed curves (*76*) for Munsell hue, value, and chroma.

(4) A descriptive name according to the I.S.C.C.-N.B.S. system of color designation (*50*, *51*, *81*) may be derived from the Munsell notation. This name is recommended wherever general comprehensibility is desired and precision is not important. The use of color names for color specification is not recommended.

F·DEFINITIONS

9·35 Color stimulus terms (physics).

Radiant flux is the rate of transfer of radiant energy (watts).

Irradiance is the areal density of incident radiant flux (watts per square meter).

Radiant transmittance is the ratio of transmitted to incident radiant flux.

Radiant reflectance is the ratio of reflected to incident radiant flux.

Spectral irradiance is the density of radiant flux incident on a surface per unit wavelength (watts per square centimeter per millimicron).

9·36 Color terms (psychophysics).

Luminous flux is the rate of transfer of luminous energy (lumens).

Luminosity is the luminous efficiency of radiant flux (lumens per watt). The luminous flux corresponding to a given radiant flux evaluated in watts may be obtained in lumens by multiplying the number of watts by the luminosity of the given radiant flux in lumens per watt.

Illuminance is the areal density of incident luminous flux (lumens per square meter, or meter candle, or lux).

Luminance of a color is the luminous flux per unit solid angle emitted per unit projected area of the light source to which the color belongs (millilamberts).

Luminous transmittance is the ratio of transmitted to incident luminous flux.

Luminous reflectance is the ratio of reflected to incident luminous flux.

Dominant wavelength of a color is the wavelength of the portion of the spectrum that, when combined with an achromatic light in suitable proportions, matches the color (millimicrons).

Complementary wavelength of a color is the wavelength of the portion of the spectrum that, when combined with the color in suitable proportions, matches the adopted achromatic light.

Purity of a color is determined by the ratio of the luminance of the spectrum component to that of the achromatic component of a mixture matching the color.

Excitation purity of a color is the fraction of the distance in the I.C.I. standard chromaticity diagram from the achromatic point to the spectrum locus or the purple boundary. Usually excitation purity is indicated by the word *purity* alone.

Tristimulus values of a color are the amounts of the three primaries required to produce a match for it. In the I.C.I. standard system the tristimulus values are designated by X, Y, and Z. The tristimulus value Y is either the luminance if the color belongs to a self-luminous area, or the luminous transmittance if it belongs to a transparent object, or the luminous reflectance if it belongs to an opaque object.

Chromaticity coordinate of a color is the ratio of any one of the tristimulus values to the sum of these three values. Any pair of these three coordinates may be used to specify the chromaticity of the color.

Munsell value of an object color is an expression of its luminous reflectance, or transmittance, on a scale giving approximately uniform perceptual steps (see Table 9·14). Munsell value of an opaque surface may be found approximately by taking the square root of the luminous reflectance expressed in per cent.

Munsell hue of an object color is a designation that may be found from its tristimulus values, X, Y, Z, or from its Y value and its chromaticity coordinates, x, y (*76*). The Munsell hue scale has approximately uniform perceptual steps; under ordinary observing conditions Munsell hue correlates well with the hue of the color perception.

Munsell chroma of an object color expresses the departure of the color from the nearest gray on an arbitrary scale defined in terms of its Y value and its chromaticity coordinates, x, y (*76*). The Munsell chroma scales have approximately uniform perceptual steps; under ordinary observing conditions Munsell chroma correlates well with the saturation of the color perception.

9·37 Color perception terms (psychology).

Hue is the attribute of a color perception that permits it to be classified as red, yellow, green, blue, purple, or an intermediate.

Chromatic color is color perceived to have a hue.

Achromatic color is color perceived to have no hue; black, gray, white, silver, and colorless refer to achromatic color perceptions.

Saturation is the attribute of a color perception that determines its degree of difference from the nearest achromatic color perception.

Brightness of a color perceived to belong to a self-luminous area is the attribute that permits it to be classified as equivalent to some member of the series of achromatic color perceptions ranging from very dim to very bright. The color perception corresponding to the clear night sky under usual observing conditions is near the bottom of this scale; that corresponding to the noon-day sun is near the top.

Lightness of a color perceived to belong to an illuminated object is the attribute that permits it to be classified as equivalent to some member of the series of gray colors ranging from black to white.

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